A physicochemical and microbial study of two meromictic lakes in South-West Tasmania, with observations on Australian Chrysophytes

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DECLARATION

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university and contains no copy or paraphrase of material previously published or written by another person, except where due reference is made in the text.

Roger Croome
1st May 1984.

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A PHYSICOCHEMICAL AND MICROBIAL STUDY OF TWO MEROMICTIC LAKES IN SOUTH-WEST TASMANIA, WITH OBSERVATIONS ON AUSTRALIAN CHRYSOPHYTES

ABSTRACT

Lake Fidler and Sulphide Pool are small, dystrophic, meromictic lakes adjacent to the Gordon River in South-west Tasmania. Partitioning between upper and lower waters is very distinct in both lakes, and a stratification of microorganisms is situated about a particularly abrupt redoxcline at around three metres depth in Lake Fidler and two metres depth in Sulphide Pool. A special close-interval sampler was developed specifically to sample the fine zonation of these organisms. The microbial stratification in Lake Fidler comprises small flagellate algae, colourless sulphur bacteria, and green photosynthetic bacteria. In Sulphide Pool a similar stratification occurs but lacks colourless sulphur bacteria. Extremely large standing crops of photosynthetic bacteria are present in the two lakes, and the algae of the stratified communities are also present in large numbers, particularly in Sulphide Pool. The organisms of the microbial arrays which photosynthesise do so at very low levels of red light. The physicochemical limnology of the lakes, structure of the microbial stratifications, and the taxonomy, ecology and physiology of the organisms within them, are described.

Many of the organisms present in the surface waters of the two lakes are Chrysophytes. This group of algae has not been studied previously in Australia, and the large number of Chrysophytes discovered in Lake Fidler and Sulphide Pool prompted an Australia-wide survey of these organisms. Three new Chrysophytes are described, and many more are reported for the first time in the southern hemisphere.

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Finally, I wish to say that I am very glad to have had the opportunity to carry out the study documented herein, and thanks for that go, in large part, to my family.

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1. INTRODUCTION

Lake Fidler and Sulphide Pool (Figs. 1 and 2) are small dystrophic water bodies adjacent to the Gordon River in South-West Tasmania. When first sampled in 1977 as part of a large scientific study in the area, they were found to have high concentrations of dissolved sulphide in their bottom waters, and were subsequently shown to be meromictic (King & Tyler, 1981b). These early studies also discovered a stratification of micro-organisms at depth in the lakes, across the redoxcline between their mixolimnetic and monimolimnetic waters.

More detailed studies (King & Tyler, 1982a, 1983) investigated the physicochemical limnology of the lakes, discussed the depth of the microbial stratifications in relation to underwater radiation, and quantified the microbial populations via chlorophyll analyses. The physicochemical features were studied further during an investigation of their meromictic stability and permanence (Bowling & Tyler, 1984).

The study documented here was designed to investigate more closely the organisms present in the lakes and their ecology, particularly those making up the microflagellate/bacterial array about the redoxcline. Twelve field trips were made from January 1981 to April 1983, ecological parameters were assessed by established techniques, and the organisms were examined by light and electron microscopy.

The work extended beyond that originally proposed because many of the organisms found in the lakes belonged to the Chrysophyceae, an algal group which had received little attention in Australia. A proper taxonomic assessment of these organisms required comparable observations from other water bodies, and the project was extended to include a preliminary survey of Australian Chrysophytes.

At the time of writing, ten papers have been prepared from the work reported herein, three concerning the organisms of the microbial stratifications of Lake Fidler and Sulphide Pool, and seven concerning Chrysophyte taxonomy and ecology (see Appendices I-X).

2. MATERIALS AND METHODS

The remoteness of the lakes presented both logistic and analytical difficulties. Concerning the latter, analytical techniques were selected or developed with an eye to the most efficient use of time and resources in the field, and to the lability of the various parameters being determined. Water temperatures, underwater light penetration, spectral distribution, and transmission were measured *in situ*. Redox potential, pH, conductivity, dissolved oxygen and bicarbonate alkalinity were determined on the day of sampling. Samples for analysis of total sulphide were fixed immediately and usually analysed within a week. Colour and turbidity were measured within a week of sampling. Chloride, sulphate and all cations were measured within a month on samples stored at $4^{o}C$. Bacterial and algal counts were usually made within a month. Samples for chlorophyll determinations were stored in light-proof bottles at around $5^{o}C$ and always received priority on return to the laboratory, usually being analysed 2-3 days after sampling. Material for ultra thin sectioning was fixed in the field and dehydrated to 75% acetone within a day of sampling. Microscopical examination of live material was usually made in the field after concentration of cells via plankton net, centrifugation or filtration.

Water temperatures were measured with a thermistor, Redox potential and pH were measured electrometrically with portable meters. Dissolved oxygen was measured by the azide modification of the Winkler method (Anon., 1971). Sulphide was determined iodometrically, after precipitation with ZnAc and NaOH, to April 1981 and thereafter by the Pachmayr method (after Brock *et al.*, 1971). Conductivity was measured using a portable meter. Bicarbonate alkalinity was determined by titration with 0.01 M HCl to pH 4.5 (Golterman, 1969) after addition of 3 mL of 2% $CdCl_2$ to suppress interference by sulphide, chloride by conductrometric titration with $AgNO_3$ (Golterman, 1969), sulphate turbidimetrically (Anon., 1971), calcium by the bis - (2-hydroxyanil) method (Kerr, 1960), and sodium, magnesium and potassium by atomic absorption spectrophotometry. Colour was determined spectrophotometrically at 440 nm (= G_{440}, Kirk, 1976). Turbidity was measured nephelometrically. Light penetration was measured using a Secchi disc. Photosynthetically active radiation (PAR) was measured with a Licor LI - 185 quantameter (400-730 nm), and spectral distribution with a Techtum QSM scanning quantaspectrometer (400-740 nm with 18 nm bandwidth). *In situ* measurements of transparency were made with a Kahl underwater turbidimeter.*

*Many of these methods were found to be suitable for Lake Fidler and Sulphide Pool by King & Tyler (1981b, 1982a, 1983), others by A.L.Baker, K.K.Baker and Tyler (unpublished).

Inorganic carbon fixation was measured using the C^{14} method of Steeman Nielsen (1952). Algal counts were made on samples fixed in 5% formalin or Lugol's Iodine, using an inverted microscope (Lund *et al.*, 1958). Bacterial counts were made on cellulose nitrate filters of pore size 0.2 μm : lake water (usually 1 or 3 mL) fixed with 5% formalin was flushed through with bacteria free water, the filters were stained in 3% erythrosin in 5% phenol for 20 hours, leached in distilled water, dried, and viewed microscopically at 1250 x after being cleared with cedar oil.

Samples taken for *in vitro* determinations of chlorophyll to April 1981 were filtered through 0.2 μm pore size cellulose nitrate filters in the field, extracted in acetone on return to the laboratory, and examined spectrophotometrically using a Pye-Unicam SP8-100 spectrophotometer. From May 1981 samples were returned to the laboratory before filtering, and were extracted in methanol for 1 hour at $4^{o}C$ in the dark. Bacterial chlorophyll levels were calculated after Jones (1979), algal chlorophyll after Golterman *et al.* (1978). Acetone was also used as a solvent for diagnostic purposes. *In vivo* spectra of algae and bacteria were determined using similar filters which were air dried and scanned after being cleared with cedar oil.

Material for direct observation by transmission electron microscopy was fixed in 1% glutaraldehyde for 30 min. or 5% formalin and placed on formvar coated copper grids with or without a coating of polylysine (Marchant & Thomas, 1983), shadowed with platinum/palladium (2 parts to 1) and viewed using an Hitachi H-300 transmission electron microscope. Material for ultrathin sectioning was concentrated by centrifugation, fixed in 1% glutaraldehyde for 30 min. and 1% osmium tetroxide for 15 min., dehydrated in an acetone series, and embedded in Spurr's resin. Sections were cut with glass knives using an LKB Ultratome Type 4801/2 A, placed on formvar coated copper grids, and viewed after staining for 10 min. in 1% uranyl acetate and 15 min. in Reynold's lead citrate. Material for scanning electron microscopy was placed on polylysine-coated glass coverslips, dehydrated in an acetone series, critical point dried using CO_2 as the intermediary fluid, sputter coated with gold, and viewed using a Philips 505 scanning electron microscope.

Details of any refinements or alterations to the above methods for specific purposes will be found in the text at the relevant place.

Twelve sampling trips were made to Lake Fidler and Sulphide Pool, from January 1981 to April 1983 - see Table 1. "Basic" denotes sampling at various depths and particularly across the redoxcline for organisms,

redox potential, dissolved oxygen, sulphide, chlorophyll, and conductivity, plus temperature and underwater light determinations.

Trip	Dates	Details of sampling
1	29 Jan - 5 Feb 1981	Basic
2	12 - 17 March 1981	Basic
3	18 - 24 April 1981	Basic
4	26 - 28 May 1981	Basic. Methanol introduced for chlorophyll extraction.
5	31 Aug - 4 Sept 1981	Basic + Transmissometry. Pachmayr sulphide method introduced
6	16 - 19 Oct 1981	Basic + Night sampling. Large sampler introduced
7	10 - 13 Dec 1981	Basic + Inorganic carbon fixation trial.
8	7 - 12 Feb 1982	Basic + Transmissometry + Night sampling
9	4 - 6 April 1982	Basic + Transmissometry + Detailed chemistry + Electron microscopy
10	3 - 6 Nov 1982	Basic + Electron microscopy
11	22 - 25 Feb 1983	Basic + Electron microscopy
12	11 - 14 April 1983	Basic + Microscopy.

Table 1. Dates and details of sampling trips to Lake Fidler and Sulphide Pool. (See text for explanation of "Basic").

When water samples from the lakes at intervals of 0.5 m or greater were required a 0.25 m long Van Dorn sampler was used, but more sophisticated samplers were needed to collect samples from the region of the microflagellate/bacterial "plate." Sampling in the vicinity of the plate by King & Tyler (1981b,1982a,1983) was done with a microsampler which took samples 15 cm apart over a depth of 1 metre (after Baker, 1970). In 1980 Baker visited Tasmania and worked with Tyler on Lake Fidler and Sulphide Pool, developing a microsampler capable of sampling 5 cm apart (Baker *et al.*, in prep.). The samplers most often used in the study documented herein (see Figs. 3 & 4) were constructed on the principles developed by Baker *et al.* The largest took 5 replicate samples at 5 cm intervals over a depth of 1.15 metres. (This was a development of a similar sampler constructed by Baker *et al.* which sampled over a depth of 0.20 metres. Such a sampler was used in this study on one occasion only - in Sulphide Pool of 15 March 1981). The sampler operated pneumatically, air being supplied or withdrawn via a small pump powered by a 12 volt motor-cycle battery. (A hand-pump was used on the first few sample trips but proved inadequate for the larger sampler). When preparing to take a sample, air was pumped into the sampler, moving the plungers forward in

60 mL plastic syringes. A positive pressure was maintained while the sampler was lowered to the required depth. The pressure was then released, air was pumped out, and the consequent negative pressure drew the plungers of the syringes back, thereby collecting water samples at discrete depths.

The largest sampler (Fig. 3) took 5 replicate samples allowing, for instance, simultaneous sampling for (1) redox potential, pH and conductivity, (2) algal and bacterial counts, (3) dissolved oxygen, (4) sulphide and (5) chlorophyll. Chemical fixation, for dissolved oxygen and sulphide for instance, was accomplished in the sampler syringes simply by injecting the required reagents via the syringe ports. The 24 syringes for any one parameter were removable in 2 racks of 12, and the 12 syringes of each rack could be evacuated simultaneously (Fig. 5), or one by one if screw-on taps were fitted after sampling.

The samplers worked well. A few syringes stuck on the large model on most samplings but this was not too inconvenient. Premature sampling also occurred occasionally in some syringes, but with such a large number of parameters being determined an examination of the results made it obvious when this had happened.

Occasionally, large volumes of water were required from a narrow depth stratum. Such sampling was accomplished by the use of a hand-pump, plastic tubing, and two funnels placed top to top (after Parker *et al.*, 1968) (see Fig. 6). Used only for qualitative work, e.g. collection of cells from the population peak of *Scourfieldia caeca*, this sampling technique was also very successful.

Some 30 people accompanied supervisor Dr. Peter Tyler and the writer to the lakes during these twelve trips, ranging from individuals interested in visiting a relatively inaccessible wilderness area, to scientists from overseas interested in seeing the unique meromictic lakes of the Gordon River at first hand. Many of these people aided in the collection of samples and/or data. It should be noted in particular that the first five trips (Jan. - Sept., 1981) were made in the accompaniment of Honours student Lee Bowling who used some of the data jointly collected for his investigations of the physicochemical limnology of the lakes, their meromictic stability and permanence, underwater light penetration, and the palaeolimnology of a third small lake in the area, Lake Morrison (Bowling, 1981).

3. RESULTS AND OBSERVATIONS

3.1 BACKGROUND INFORMATION

Lake Fidler and Sulphide Pool are two of four small lakes adjacent to the Gordon River in South-west Tasmania (Figs.1,2,7). They are unusual lakes in many respects, but primarily in that they are meromictic, with dystrophic surface waters of low salinity ($K_{18} \simeq$ 100 µS/cm) and bottom waters of estuarine origin (K_{18} 1500-4500 µS/cm). Lake Morrison, some 10 km downstream, was also meromictic when first studied in 1977, but has since become holomictic, most probably because of alteration of the flow regime in the Gordon River due to impoundments built upstream for hydro-electric power generation. Lake Fidler and Sulphide Pool are likely to become holomictic also, as both the volumes and salinity of their respective monimolimnia are decreasing.

Lake Fidler, Sulphide Pool, and Lake Morrison are considered to be levee lakes, being formed behind accumulations of silt deposited by the river. The fourth water body, Perched Lake, has its surface approximately 17 metres above that of the river. Of unknown origin, it is moderately dystrophic and not meromictic.

Several papers have already been published on limnological studies of the four lakes and the adjacent river (King & Tyler, 1981a:b,1982a:b, 1983, Croome & Tyler, 1983b:c), and more are being prepared (e.g. Bowling & Tyler 1984).

The climate of the area, and the vegetation around the lakes, has been detailed by King & Tyler (1981b). The climate is distinctly maritime, under the influence of prevailing westerly winds. Observations at the nearest meteorological stations (Strahan, 55 km to the north-west, and Strathgordon, 41 km to the east) suggest that the annual rainfall in the vicinity of the lakes is between 1750 and 2580 mm, and the annual temperature range (based on monthly means) is 3-21°C or thereabouts. The lakes are situated in dense rainforest dominated by mixtures of trees, principally *Nothofagus cunninghamii* (Hook.) Oerst, *Lagarostrobos franklinii* (Hook. f.) Quinn, *Phyllocladus asplendiifolius* (Labill.) Hook. f., *Anadopetalum biglandulosum* A. Gunn ex Hook. f., and *Acradenia franklinii* Milligan ex Kippest. Lake Fidler and Sulphide Pool are fringed by a narrow zone of rooted macrophytes, principally *Triglochin procera* R.Br. and *Baumea rubiginosa* (Spreng.) Boeck., outside which is a herbfield comprising such species as *Nymphoides exigua* (F. Muell.) Kurtze, *Liliopsis brownii* A.W. Hill and *Ranunculus rivularis* Banks & Sol. ex D.C. The herbfields, which give way to scrub or forest, are inundated when lake levels rise.

Morphometric data for Lake Fidler and Sulphide Pool, the two water bodies on which this study concentrates, are given in Table 2, while lake bathymetry and the locations of inflows and outflows are shown in Figure 8.

Parameter	Lake Fidler	Sulphide Pool
Area (ha)	1.28	0.11
Volume (m^3)	41317	1727
Maximum depth (m)	7.6	2.7
Mean depth (m)	3.2	1.5
Length (m)	186	78
Width (m)	104	24
Shoreline length (m)	540	165
Shoreline development	1.3	1.4

Table 2 Morphometric parameters of Lake Fidler and Sulphide Pool (after King and Tyler, 1981b).

Lake Fidler is by far the larger and deeper of the two lakes, and has two inflows from its own catchment and one inflow-outflow from the Gordon River. Its level fluctuates (with a lag of an hour or so) with that of the river. Sulphide Pool, sited in a flatter area than Lake Fidler, has no obvious inflows from its surrounds, but again there is an inflow-outflow creek to the Gordon River, and water levels fluctuate with those of the river. Changes in level of up to two metres can occur in both lakes (P. Tyler, pers. comm.). The buoy locations in Figure 8 are those used most often in routine sampling; other temporary buoys were used from time to time if, for instance, disturbance was suspected or if replicate samples from various parts of the lake were needed.

The first regular observations of Lake Fidler and Sulphide Pool were made by King and Tyler (1982a, 1983) from January 1977 to April 1978. Their work was comprehensive, thoroughly investigating the physico-chemical limnology of the lakes and making preliminary observations of their biota.* The work documented herein, for the period January 1981 - April 1983, is essentially an investigation of the organisms inhabiting the lakes. To this end, a considerable amount of ecological data are presented. The data were collected primarily to investigate the environment of the organisms but they also serve a second purpose, allowing a comparison to be made between the physicochemical conditions in the lakes in 1977-78 and those prevailing in 1981-83.

* The lakes were further investigated by A.L.Baker, K.K.Baker and Tyler in 1980, but the results of this work remain unpublished and were not made available to the writer.

3.2 "WHOLE-DEPTH" PROFILES

The physicochemical data can be divided in two: that collected over the entire lake depth, and that collected at discrete depths using the close-interval sampler. The latter will be presented later with the biological data collected in the same manner; the data collected over the entire lake depth are presented below.

3.2.1 Lake Fidler (Figs. 9-18)

During this study the surface water temperatures of Lake Fidler varied from 9.3-23.8°C, while those of the monimolimnion were 10.4-11.8°C. In summer the thermal profile of the lake is often typical of a shallow holomictic water body (e.g. Fig. 9). In winter, however, the profile reflects the meromictic nature of the lake, with a temperature difference of 2-3°C between waters of the mixolimnion and the warmer monimolimnion (e.g. Fig. 13). There is often a temperature increase of up to 1°C at or just below the redoxcline (e.g. Figs. 11 and 18), producing a distinct mid-water bulge in the thermal profile. These temperature bulges correspond to the depth of high concentrations of the photosynthetic bacteria which have done much to arouse scientific interest in Lake Fidler. During this study the temperature range at the depth of these photosynthetic bacteria was 8.1-13.3°C, considerably less than that observed at the surface.

The surface waters are well aerated, and the bottom waters heavily sulphuretted. Figure 16 typifies these parameters: surface oxygen values of 6-10 mg/L persist to 2-3 metres depth (depending on lake level), then quickly decline to zero within 0.5 metres. Below this, dissolved sulphides are present, increasing to 40 mg/L within 0.5 metres. Such values may persist to the bottom of the lake, but values as high as 150 mg/L have also been recorded in bottom waters (King and Tyler, 1982a).

Concomitant with the change from oxygenated to sulphuretted waters there is a large change in the redox potential (e.g. see Figure 3). In Lake Fidler changes of up to 450 mV (positive to negative) occur within 5 cm across the dissolved oxygen/sulphide boundary. Considerable problems were experienced during this study in the measurement of redox potential. Electrode poisoning often occurred, for instance, but use of double junction electrodes would have meant the re-designing of the whole sampling programme. Hence, emphasis is placed herein on the magnitude of the changes in redox potential rather than on absolute voltage values. This has come about due to the possible inaccuracy of the measurements and, as King and Tyler (1982a) have pointed out, the uncertainty in any case of

their interpretation in thermodynamic terms (Stumm, 1967; Whitfield,1969).

From one day to the next the depth of this abrupt redoxcline is constant when related to a set datum, but varies when related to the lake surface, as the latter rises and falls with the level of the adjacent river. During this study the cline was usually at around 3 metres depth, but did vary from 2.6-3.2 metres. It is probably never less than 2.6 metres deep as the lake surface ceases to be affected by river levels below a certain river height, but is probably at depths greater than 3.2 metres from time to time as floods can raise the lake (and river) level by 2 metres or so (P. Tyler, pers. comm.).

Despite the fact that the redoxcline is found at a constant depth from day to day relative to a set datum, it did in fact deepen slightly over the period of this study, dropping by approximately 0.20 metres. This is part of a large amount of evidence that the meromictic nature of the lake is threatened (see below).

Lake Fidler, in common with most waters in south-west Tasmania, is highly dystrophic, the waters appearing dark brown due to the presence of humic materials derived from the catchment. When measured as an optical absorbance at 440 nm (G_{440} m^{-1}, see Kirk, 1976, who gave the name 'gilvin' to this dissolved organic material) values of 3.65-8.23 m^{-1} were obtained (see Table 3). Routine measurements of gilvin were reported by King and Tyler (1982a) for the period Jan. 1977 - Aug. 1978. While the two sets of data agree for the monimolimnion of Lake Fidler, the values reported here for surface waters (3.6-3.7 m^{-1}) are significantly less than those reported by King and Tyler (5-8 m^{-1}). Such differences indicate a real change in the surface waters of the lake (see below).

Turbidities are usually very low in the mixolimnion but increase markedly at 3-4 metres depth due to the zone of microorganisms across the redoxcline, and persist at lower levels in the monimolimnion.

Surface waters are acidic, as is typical of the dystrophic waters in this area (Buckney and Tyler, 1973a), and pH values are usually 6.0-6.5. There is a change in pH across the redoxcline and values in the monimolimnion are neutral to slightly basic.

Electrical conductivities were determined from lake surface to bottom on six occasions, and on one of these (4 Apr. 1982) detailed analyses were made of the major ions present (see Table 3). The profiles are typified by that of 18-21 Apr. 1981 (Fig. 11); conductivities of around 100 μScm^{-1} (at 18°C) to a depth of two metres, a steep rise in conductivity between 2.5-3.5 metres, and conductivities of 4500-5000 μScm^{-1} in the bottom

3-4 metres.

King and Tyler (1982a) reported similar values for the monimolimnion of Lake Fidler, but usually found much higher conductivities in the surface waters than those reported here. Surface conductivities during their study were usually 300-500 μScm^{-1} (at $18^{o}C$), decreasing below 300 μScm^{-1} on two occasions only. During this study the maximum surface value found was 117 μScm^{-1} and values such as this persisted to around a depth of 2.0 metres. (King and Tyler regularly reported values around 1500 μScm^{-1} at 1.0 metres). The decline of surface water salinity is consistent with the decline in surface salinity of the adjacent Gordon River, where dams built upstream for hydro-electric power generation have altered the flow regime to the extent that seawater intrusions up the river have been greatly reduced. This factor is in fact threatening the whole meromictic nature of Lake Fidler (see below).

Major ion analyses are presented in Table 3 and Figure 27. Sodium and chloride are by far the dominant ions, the ionic dominance for surface waters being $Na>Ca\geq Mg>K$, $Cl>HCO_3>SO_4$ and for bottom waters $Na>Mg>Ca>K$, $Cl>HCO_3>SO_4$. When plotted in ternary diagrams (Fig. 27) ionic proportions show a movement away from World Average Freshwater towards seawater with depth, illustrating the estuarine origin of most of the dissolved ions.

The results are in accord with those of King and Tyler (1982b) with three exceptions:-

(1) ionic proportions of the surface waters are closer to those of World Average Freshwater than in the earlier study (this accords with their lower electrical conductivity).

(2) the calcium values recorded here for the bottom waters are some 50 per cent higher than those recorded by King and Tyler (most probably an analytical variable).

(3) bicarbonate values are higher than those of King and Tyler, despite the fact that pH values are lower, and that King and Tyler did nothing in their analysis to suppress interference by dissolved sulphides (King, pers. comm.). (Bicarbonate values reported here were obtained after the addition of 3 mL of 2% $CdCl_2$ to 50 mL of sample: if $CdCl_2$ was not added the values obtained were up to 70 per cent higher).

Chlorophyll values in the mixolimnion of Lake Fidler (e.g. Fig. 15), and the paucity of material in plankton tows, indicate that the standing crop in this portion of the lake is low. However, chlorophyll values increase markedly in the region of the redoxcline, both Chlorophyll *a*

LAKE FIDLER

Depth (m)	K_{18} (μScm^{-1})	meq/L Na	K	Ca	Mg	HCO_3	Cl	SO_4	pH	G440 (m^{-1})	Turb. (N.T.U.)
Sur	76.1	0.43	0.03	0.17	0.18	0.18	0.50	0.04	6.5	3.65	<1
0.5	73.1	0.46	0.03	0.17	0.18	0.20	0.50	0.04		3.65	<1
1.0	74.6	0.43	0.03	0.19	0.18	0.19	0.50	0.04	6.5	3.70	<1
1.5	77.8	0.47	0.03	0.19	0.18	0.20	0.52	0.04		3.70	<1
2.0	95.8	0.60	0.03	0.19	0.18	0.20	0.70	0.05	6.35	3.60	<1
2.5	296	2.46	0.11	0.28	0.61	0.30	2.5	0.10	6.2	4.18	<1
3.0	1410	10.7	0.44	2.28	2.6	1.84	12.2	0.39	6.8	7.33	4
3.5	3420	26.8	0.91	3.85	7.0	3.64	31.8	1.05	7.0	8.23	23
4.0	4050	32.7	1.32	4.15	8.4	3.94	40.4	1.63	7.0	7.45	27
4.5	4250	35.8	1.41	4.15	9.0	4.10	41.1	1.69	7.0	7.75	3
5.0	4340	36.5	1.45	4.15	9.2	4.10	43.6	1.63	7.0	7.25	8
5.5	4430	36.5	1.45	4.15	9.5	4.10	43.7	1.49	7.0	7.15	9
6.0	4420	36.5	1.46	4.15	9.5	4.60	43.7	1.55	7.0	7.20	4
6.5	4520	38.3	1.53	4.25	9.9	4.56	45.6	1.57	7.0	7.15	28
7.0	4560	38.3	1.53	4.25	9.9	4.80	46.0	1.34	7.0	6.93	12

SULPHIDE POOL

Depth (m)	K_{18} (μScm^{-1})	meq/L Na	K	Ca	Mg	HCO_3	Cl	SO_4	pH	G440 (m^{-1})	Turb. (N.T.U.)
Sur	93.2	0.63	0.04	0.12	0.20	0.16	0.74	0.02	5.15	9.25	<1
0.5	93.8	0.68	0.03	0.13	0.20	0.16	0.74	0.02	5.3	9.50	<1
1.0	95.0	0.68	0.03	0.13	0.20	0.18	0.75	0.02	5.3	9.50	<1
1.5	193	1.52	0.06	0.19	0.39	0.28	1.5	0.06	5.5	9.75	1.4
2.0	318	2.68	0.11	0.28	0.65	0.60	2.8	0.09	5.8	9.75	1.1
2.5	1410	10.7	0.47	0.87	2.58	1.42	12.2	0.25	5.9	8.78	5.4

Table 3. Major ion and other physicochemical analyses of Lake Fidler and Sulphide Pool on 4 April 1982.

and Bacteriochlorophyll *d* being detected in large amounts. The high levels of Bacteriochlorophyll *d* persist into the mixolimnia. As an investigation of these chlorophyll levels and the organisms associated with them form the major part of this thesis, further discussion on this aspect of the work will be left until later.

Vertical Attenuation Coefficients are high (0.9-1.7 m^{-1}) and Photosynthetically Active Radiation (PAR) is rapidly attenuated by the dystrophic waters (Figs. 9-18, and Fig. 28). Less than 20 per cent of PAR incident on the lake surface reaches a depth of 0.5 metres, 1 per cent or less reaches a depth of 2.0 metres, and no light at all can be detected below about 3.2 metres. As the redoxcline is regularly at a depth of 2.6-3.2 metres this attenuation of light has important consequences for the photosynthetic microorganisms astride the dissolved oxygen/sulphide boundary, all of whom receive much less than 1% of surface PAR.

In addition, it is the shorter wavelengths which are more rapidly attenuated (Fig. 29). Hence, at a depth of 1.0 metres almost all light below 500 nm has been quenched, and by 2.8 metres only a small amount of light in the range 560-700 nm can be detected.

On the day the spectra in Figure 29 were determined green photosynthetic bacteria were present in significant numbers below 2.3 metres, with a population peak at 2.65 metres. An *in vivo* spectrum of photosynthetic bacterial cells which was determined in the laboratory (see later) has been drawn on Figure 29 to show the relationship between the absorption characteristics of the cells and the light present at depth in the lake.

Most underwater light measurements were made in calm sunny conditions, so that extinction coefficients and the underwater light profile could be ascertained accurately. Measurements made in cloudy or overcast conditions were difficult to assess but gave a profile of similar shape, showing that light reached equivalent depths but was reduced in intensity.

The penetration of light into Lake Fidler was greater during this study than that measured by King & Tyler. This is in accord with the reduced gilvin levels of the surface waters and is an important difference between the lake as it was in 1977-78 and as it is now.

Overall, the above observations show important changes in the physicochemical limnology of Lake Fidler since the observations of King and Tyler. Of major significance is a deepening of the mixolimnion, with a drop of around 1.0 metre in the redoxcline. This deepening is continuing, a further drop in the redoxcline of around 0.20 metres being noticed during this study. At the same time the mixolimnetic waters have become less saline and less highly coloured. Photosynthetically active radiation now penetrates more deeply into the waters of the lake, but the drop of around 1.0 metre of the redoxcline means that the organisms about it receive light of comparable intensity and quality to that measured by King and Tyler.

The changes outlined above are in accord with the hypothesis that alterations in the flow regime of the Gordon River have reduced the incursion upstream of the estuarine water responsible for maintaining the meromictic condition of the lake (Bowling and Tyler, 1984). Unless river flows are manipulated to allow salt water to penetrate sufficiently far upstream, the mixolimnion will continue to deepen and the lake will eventually become holomictic.

3.2.2 Sulphide Pool (Figs. 19-26)

During this study surface temperatures ranged from 10.4-31.0^{o}C, while those of the monimolimnion were 8.3-11.8^{o}C. Thermal profiles show a variety similar to that of Lake Fidler, but are different in being much steeper near the surface due to the more highly coloured waters and to the more sheltered nature of the lake. On one memorable occasion (2 Feb. 81, Fig. 19), while smoke from forest fires passed overhead, a temperature of 31^{o}C was recorded at the surface, while that recorded at a depth of 0.5 metres was 21.8^{o}C.

Again, a distinct temperature bulge is occasionally detected at or just below the redoxcline (e.g. Fig. 25) where the temperature variation recorded for the period of the study was 7.0-13.7^{o}C.

Dissolved oxygen values in the mixolimnion are more variable than those of Lake Fidler. Typically, they are at a maximum near the surface and decrease with depth to zero at around 2.0 metres, e.g. Figure 24. However, the surface maximum can vary from around 2 mg/L to 6 mg/L or more between successive days, e.g. Figure 25, and occasionally oxygen is depleted at depths above 2.0 metres, so that dissolved oxygen 'bulges' are present, e.g. Figure 19.

Below the oxygenated waters dissolved sulphides appear, increasing to around 15 mg/L within one metre.

As with Lake Fidler, there is a drop in redox potential across the dissolved oxygen/sulphide boundary, but in Sulphide Pool the drop is usually less distinct and is sometimes stepped, e.g. Figure 45. From day-to-day, as lake levels rise and fall, the redoxcline maintains a discrete depth relative to a set datum. However, it did drop significantly over the period of the study, the fall being approximately 0.50 metres, considerably more than the fall of around 0.20 metres recorded in Lake Fidler. And it has also fallen significantly since the study of King and Tyler who regularly recorded it at around 1.0 metres below the surface on a day-to-day basis. (During this study it varied from 1.5-2.1 metres below the surface on a day-to-day basis). Such a rapid deepening of the redoxcline suggests the early demise of the meromictic condition in Sulphide Pool.

Sulphide Pool is more highly coloured than Lake Fidler, with G440 values of around 9 m^{-1} (Table 3). These values are slightly less than the surface values reported by King and Tyler (1983) but slightly greater than their monimolimnetic values, again evidencing a real change in the nature of the lake.

Turbidities are low in the surface waters but do increase in the monimolimnion (Table 3).

pH values in the surface waters of Sulphide Pool have been as low as 3.8 (King and Tyler, 1983) and during this study were usually around 5.0. Values increased across the redoxcline to around 6.0 (maximum value of 6.3), but in general pH values in Sulphide Pool are much lower than those of Lake Fidler.

Electrical conductivities are graphed for three occasions (Figs. 22, 24 and 25), major ions being analysed in detail on one of these (4 Apr. 1983 - see Table 3). The profiles are typified by that of 10 Feb. 1982 (Fig. 24), with conductivities of around 100 μScm^{-1} to a depth of one metre, and a gradual increase to around 1600 μScm^{-1} at 2.5 metres. The surface salinities agree with those recorded by King and Tyler, but those near the bottom are around 1000 μScm^{-1} less than those at equivalent depth in 1977-78. There has clearly been a marked leeching of salt or a flushing of saline water from the monimolimnion of Sulphide Pool, and it is apparent that the lake may soon become holomictic.

Major ion analyses (Table 3, Fig. 27) show similar ionic dominance and proportions to Lake Fidler, all waters being close to seawater, and again

the values are similar to those reported by King and Tyler (1982b).

As would be expected from the gilvin values, Vertical Attenuation Coefficients are higher (1.5-2.5 m^{-1}) and light is attenuated more rapidly in Sulphide Pool than in Lake Fidler (Figs. 19-26, and Fig. 28): less than 10 per cent of surface PAR reaches a depth of 0.5 metres, 1 per cent or less reaches 1.2 metres, and usually no light is detectable below 2.0 metres. Much less than 1 per cent of surface PAR reaches the depth of the redoxcline.

The shorter wavelengths are also more rapidly attenuated in Sulphide Pool. The spectra at various depths are shown in Figure 30. At a depth of 0.5 metres almost all light below 500 nm has been quenched, and by 1.6 metres only a small amount of light is present, at wavelengths greater than 580 nm.

On the day the spectra in Figure 30 were determined there were significant numbers of green photosynthetic bacteria below 1.3 metres, but in addition there were large numbers of green flagellate algae present, with a population peak around 1.4 metres. An *in vivo* spectrum of the algal cells determined in the laboratory (see later) has been drawn on Figure 30, and it is readily apparent that the troughs observed in the spectra at 1.4 and 1.5 metres were due to absorption of red light by these algae.

Secchi depths are equivalent to the depth at which 0.5-1.1 per cent of surface PAR is recorded.

Chlorophyll values in the mixolimnion of Sulphide Pool are generally higher than those of Lake Fidler (e.g. Fig. 23). Algal counts of these waters were made from time to time (see later) and significant numbers of several species of algae were detected, including a new species of Chrysophyte *Mallomonopsis tasmanica* Croome and Tyler, 1983. Further discussion of these aspects of the work will be left until later.

Alterations to the flow regime in the Gordon River have had a greater impact on Sulphide Pool than Lake Fidler. There has been considerable loss of salt from the monimolimnion and a fall of around 0.50 metres in the redoxcline during this study. A large proportion of this fall (0.30 metres) occurred during 1982, possibly as a result of floodwaters flushing the lake. It is possible that by this time such flushing has re-occurred and Sulphide Pool is holomictic.

3.3 "CLOSE-INTERVAL" PROFILES

Samples were taken by close-interval samplers across the redoxcline in both lakes, this zone having been shown during earlier work to contain a complex array of microorganisms. The large amount of data collected is difficult to present. Any one sampling with the large close-interval sampler could result in the determination of 4 or 5 physicochemical parameters and counts of 6 or 7 different microorganisms at each of 24 depths only 5 cm apart. The data collected in any one lake on any one day are displayed on three graphs (Figs. 31-41, 44-55), one showing the physicochemical parameters determined, together with chlorophyll values, and the other two showing the numbers of the particular microorganisms present. The three graphs are presented side by side with a broken line across them to indicate the depth of the redoxcline. The figures have been drawn for ease of interpretation, and the biological data are presented with varying scales on the x axis. As a consequence of this, the biological data are not directly comparable from month to month. However, when it is necessary to have a direct comparison between samplings, e.g. for a replicate series or for a comparison of day and night data, the scales have been made the same.

The depth of sampling, relative to the redoxcline, was varied during the study to allow an appreciation of community structure over a greater depth than the 1.15 metre length of the sampler. On some days emphasis was placed on the organisms above the redoxcline and the sampler was operated with the redoxcline at a depth corresponding to around two-thirds of the sampler's length, on others emphasis was placed more on the photosynthetic bacteria below the redoxcline and the sampler was operated with the depth of the redoxcline closer to one third of its length. However, the microbial stratification occurs over such a small depth range that most samplings made covered the whole array of microorganisms present.

The large amount of data is presented in three parts. Firstly, a summary is given of the physicochemical parameters across the redoxcline. Secondly, the microbiological data are summarized. Thirdly, there is a detailed presentation of the individual close-interval samplings. The organisms themselves are dealt with individually in detail in Section 3.4.

3.3.1 Summary of Physicochemical Parameters

3.3.1.1 Lake Fidler (Figs. 31-41)

The chemocline between the mixolimnion and monimolimnion in Lake Fidler is evidenced by changes in several of the parameters determined: dissolved oxygen, dissolved sulphides, pH, electrical conductivity, and redox potential. Redox potential has been chosen to mark the division because of its graphic nature and the relative ease of its determination in the field.

The change in redox potential over the 1.15 metre length of the sampler was usually around 400 mV, though changes as low as 240 mV and as high as 515 mV were measured (Figs. 36 and 39 respectively). By far the largest proportion of these changes occurred within one or two sampler syringes (0.05-0.10 metres depth). On 6 April 1982 the change was particularly marked, the redox potential dropping 335 mV within 0.05 metres (Fig. 40). The change was also very abrupt on 11 Feb. 1982, with a drop of 450 mV within 0.05 metres (Fig. 39), but the other data on this sampling occasion suggest that this may in fact have resulted from an error in the determination of the redox potential at 2.75 metres. On other occasions the change was less precipitate and the redox profile was of a different appearance: on 2 Sept. 1981 (Fig. 35) the potential was constant to a depth of 3.05 metres, then declined gradually over the next 0.40 metres to a value lower by some 380 mV. Occasionally, a stepping was found in the profile: on 17 Oct. 1981 (Fig. 37) a drop of 50 mV was recorded 0.20-0.25 metres above the main drop in redox potential. (This was not a pH effect as the E_7 profile was similar). Figure 42 has been drawn to illustrate the different types of redox profile found in the lake.

A pH cline is also present at the mixolimnion/monimolimnion boundary, values increasing by 0.8-1.5 units across it. The values sometimes begin to increase at the redoxcline, e.g. 31 Jan. 1981 (Fig. 31), and sometimes up to 0.25 metres above it, e.g. 6 April 1982 (Fig. 40). The most marked pH cline was found on 11 Feb. 1982 (Fig. 39) where pH values increased from 5.6 to 7.1 within 0.20 metres, the largest part of this increase (1.1 units) occurring within 0.05 metres, concomitant with the first drop in redox potential. (The Eh value at 2.75 metres is taken to be an error). A less marked example of the shift in pH can be seen in Figure 37.

During this study dissolved oxygen concentrations 0.6 metres above the redoxcline varied from less than 1.0 to 7.5 mg/L, but always decreased

to zero at the redoxcline. When values near the top of the profile were high there was either a gradual decrease with depth, e.g. 16 Oct. 1981 (Fig. 36), or a more rapid decrease near the top of the profile, with oxygen concentrations persisting at very low levels in the 0.25 metres or so above the redoxcline, e.g. 31 Jan. 1981 (Fig. 31). The term 'micro-aerophilic zone' is given to this area of low dissolved oxygen just above the redoxcline. On occasion the microaerophilic zone extended to 0.6 metres above the redoxcline, e.g. 2 Sept. 1981 (Fig. 35). Examples of the various dissolved oxygen profiles measured in the lake are shown in Figure 43.

Dissolved sulphides increased from zero at the redoxcline to 25-40 mg/l 0.7 metres below it. The increase was usually more or less linear, e.g. 16 Oct. 1981 (Fig. 36), but occasionally there was a persistence of low concentrations for 0.20 metres or so below the redoxcline, e.g. 2 Sept. 1981 (Fig. 35). These profiles have been added to those of dissolved oxygen in Figure 43.

Dissolved oxygen and sulphides were found at the same depth on one occasion only, the 12th of April 1983 (Fig. 41), when dissolved oxygen was found down to 0.20 metres below the redoxcline. On this occasion the sampler was operated incorrectly, and was not moved forwards before being activated. Water entrained by the instrument was taken into the syringes on sampling, leading to incorrect values for dissolved oxygen and other parameters. (Such mistakes are readily detected when all the data from a close-interval sampling are analysed, the electrical conductivity of the samples being the most helpful indicator). On all other occasions, dissolved oxygen and dissolved sulphides were mutually exclusive, and on approximately half the sampling occasions there was a sample at the redoxcline in which neither parameter was detected, e.g. 2.75 metres on 16 Oct. 1981 (Fig. 36).

3.3.1.2 Sulphide Pool (Figs. 44-55).

The changes in redox potential over the 1.15 metre length of the sampler in Sulphide Pool were more variable than those seen in Lake Fidler. Some were similar to the 'typical' Fidler profile, with more or less constant Eh values at the top of the profile, a sharp drop in Eh over 0.05 or 0.10 metres and a gradual decline thereafter, e.g. 2 Feb. 1981 (Fig. 44). Others showed a marked 'stepping', e.g. 14 March 1981 (Fig. 45), where Eh values declined some 0.40 metres above the main drop in redox

potential. While most of the profiles showed a drop in potential of 400-500 mV from top to bottom, on two occasions, 18 Oct. 1981 and 24 Feb. 1983 (Figs. 48 and 55 respectively), the changes were much less: on 18 Oct. 1981 a drop of 160 mV was observed and on 24 Feb. 1983 the drop was only 100 mV. The latter profile shows a drop of only 60 mV across the redoxcline. This is the smallest change ever recorded for these lakes, and contrasts markedly with the change of 360 mV within 0.05 metres seen on the previous sampling trip (5 Nov. 1982, Fig. 54). Figure 56 has been drawn to illustrate the different types of redox profile found in the lake.

The pH cline, a constant feature of Lake Fidler, is sporadic in occurrence in Sulphide Pool. On approximately half the sampling occasions there was simply a gradual increase in pH with depth, e.g. 14 March 1981 (Fig. 45), values increasing from around 5.5 to around 6.0. One interesting variation to this type of profile was observed on 5 April 1982 (Fig. 53) when a decrease in pH of 0.2 units was apparent over the 0.30 metres immediately above the redoxcline. Other profiles showed an increase in pH in the region of the redoxcline similar to that observed in Lake Fidler, e.g. 24 April 1981 (Fig. 46), where pH increased by 1.2 units within 0.40 metres. The pH increase typically began 0.20-0.30 metres above the redoxcline in such profiles.

Dissolved oxygen values in the half metre or so above the redoxcline in Sulphide Pool were usually less than 2 mg/L. (The exception to this occurred on 5 April 1982, Fig. 53, when values of 5-7 mg/L were measured to within 0.25 metres of the redoxcline). At times low values persisted throughout the profile, e.g. 5 Nov. 1982 (Fig. 54), while on other occasions, e.g. 14 March 1981 (Fig. 45), a distinct microaerophilic zone was observed in the 0.30 metres or so above the redoxcline, and above this dissolved oxygen values increased in the manner often seen in Lake Fidler. Examples of the various dissolved oxygen profiles measured in the lake are shown in Figure 57.

Dissolved sulphide values increased more or less linearly with depth below the redoxcline, reaching 5-15 mg/L within 0.60 metres. These values are significantly lower than those measured at a corresponding depth in Lake Fidler, where concentrations of dissolved sulphides were in the range 25-40 mg/L. Typical dissolved sulphide profiles are shown in Figure 57.

Dissolved sulphides were measured in Sulphide Pool on six occasions. On three of these, 3 Sept. 1981, 18 Oct. 1981 and 5 Nov. 1982 (Figs. 47, 48 and 54 respectively), the separation between dissolved sulphides and

dissolved oxygen was as distinct as that seen in Lake Fidler, and on two of the three occasions one or two samples at the redoxcline contained neither dissolved sulphides nor oxygen. On the other three occasions, however, samples near the redoxcline were found to contain both dissolved oxygen and dissolved sulphides. On 13 Dec. 1981 (Fig. 49) dissolved sulphides and oxygen were detected together across three sampling syringes. No oxygen was found below the redoxcline but dissolved sulphides were measured up to 0.15 metres above it. (The two samples for sulphide above this were lost in sampling so sulphide could have been present even higher up the profile). Although no electrical conductivities were measured on this occasion to test the heterogeneity of the water column, the other data collected indicated that the sampling was made in an undisturbed system, and support the finding of dissolved oxygen and sulphides at the same depth. On 10 Feb. 1982 (Fig. 50) the dissolved sulphides and oxygen profiles overlapped by one syringe. The value for dissolved oxygen in this overlap, twice that of the sample above it, suggests the possibility of a sampling error and the evidence here of the two parameters occurring at the one depth is flimsy. The other simultaneous occurrence of the two parameters is certainly erroneous: on 5 April 1982 (Fig. 53) electrical conductivities and other parameters showed that the region of the redoxcline was disturbed at the time of sampling. Hence, a true overlap in dissolved sulphides and oxygen was found on only one occasion in either Lake Fidler or Sulphide Pool: in Sulphide Pool on 13 Dec. 1981.

3.3.2 Summary of Microbiological Parameters

The redoxcline zone of the two lakes is constantly populated by an array of microorganisms which maintain discrete strata. The most significant of these microorganisms are dealt with one by one in Section 3.4, and the following is a summary only of the identity of the organisms and their distribution within the lakes.

3.3.2.1 Lake Fidler (Figs. 31-41)

The most numerous organisms in the microstratification about the redoxcline in Lake Fidler are green photosynthetic bacteria of the *Chlorobium* type (Figs. 65-69). The bacteria are of three forms: rod shaped cells of *Chlorobium limicola* type, curved cells of *Chlorobium vibrioforme* type, and convoluted chains of slightly curved cells. They are present in sufficiently high numbers (up to 50 x 10^6 cells/mL) to

colour the water bright green, and give Bacteriochlorophyll values of up to 2,360 µg/L (e.g. Fig. 32). The population maximum usually occurs immediately below the redoxcline, at the level at which dissolved sulphides are first detected, but cells also occur in significant numbers in the oxygenated water some distance above the redoxcline.

Another "organism" containing Bacteriochlorophyll is the consortium "*Chlorochromatium aggregatum*" (Figs. 95-109), an ectosymbiotic association in which green photosynthetic bacteria envelope a large, colourless, motile bacterium of uncertain affinity. On any one sampling the population peak of the consortium may be above, below, or astride the redoxcline, as it is highly motile and able to select different depths. Concentrations of up to 600,000 consortia/mL have been determined.

The most numerous alga present is the minute green flagellate *Scourfieldia caeca* (Figs. 77-94). It is frequently present above the redoxcline in an extremely thin stratum (0.05-0.10 metres) at concentrations up to 700,000 cells/mL or more, giving Chlorophyll *a* values up to 850 µg/L. Other algae which maintain a distribution above the redoxcline on a permanent or occasional basis are *Trachelomonas volvocina* (Figs. 120-122) in numbers up to 2,750 cells/mL, *Cryptomonas* sp. at up to 350 cells/mL, and *Synura petersenii* at up to 3,600 cells/mL. From time to time other algae are present at all depths in the profile; two diatoms, *Rhizosolenia eriensis* and *Fragilaria ulna*, are seen occasionally, the former in concentrations up to 30,000 cells/mL. The chlorophyte *Ankistrodesmus falcatus* var. *mirabilis* also occurs intermittently, at concentrations up to 760 cells/mL.

The colourless sulphur bacterium *Beggiatoa* is a more or less constant member of the microstratification, being present in highest numbers (up to 30,000 filaments/mL) above the redoxcline, but also being seen in significant numbers below it. A similar distribution is shown by a large, non-pigmented, motile bacterium of unknown affinity which is present in numbers up to 140,000 cells/mL. The organism has not been positively identified, but has been allied to *Achromatium* (see Figs. 110-119).

Other bacteria are observed from time to time (Figs. 126-132) particularly in the first sampling syringe below the redoxcline and associated with the first appearance of dissolved sulphides.

A detailed discussion of the most significant organisms, which includes their taxonomy, previously known ecology, and distribution in Lake Fidler, is given in Section 3.4.

3.3.2.2 Sulphide Pool (Figs. 44-55)

In the more sheltered, pond-like Sulphide Pool there are blooms of microorganisms from time to time in the surface waters, and occasionally a stratification of organisms can be demonstrated therein. However, the most significant stratification of microorganisms again occurs about the redoxcline.

The most numerous organisms are again green photosynthetic bacteria of the *Chlorobium* type. The population is less sharply defined than in Lake Fidler, but again is usually at a maximum at the depth of the redoxcline and the first occurrence of dissolved sulphides. Three forms are again present: rod-shaped cells, curved cells, and filaments of curved cells (Figs. 73-76). The filaments are different to those in Lake Fidler in that they are made up of cells lying in the one plane.

The bacterial consortium "*Chlorochromatium aggregatum*" also occurs in Sulphide Pool (as from April 1982), but is more evenly spread throughout the microstratification than in Lake Fidler.

Flagellate algae play a greater role in the microstratification than in Lake Fidler, and at times Chlorophyll *a* concentrations above the redoxcline exceed those of Bacteriochlorophyll *d* below it. *Scourfieldia caeca* and *Cryptomonas sp.* are the dominant flagellates, but *Trachelomonas volvocina, Euglena sp.*, and other species appear occasionally.

Other organisms observed from time to time are *Ankistrodesmus falcatus* var. *mirabilis, Mallomonopsis tasmanica,* described as a result of this study with Sulphide Pool as its type locality, and several unidentified bacteria. *Beggiatoa* and *cf. Achromatium,* both prevalent in Lake Fidler, are not found in Sulphide Pool.

3.3.2.3 Chlorophyll

The very first data collected from Lake Fidler (Fig. 31) illustrated well many of the features discussed above: a sharp drop in redox potential, a pH cline, decrease in dissolved oxygen levels with depth to a distinct microaerophilic zone, and a linear increase of dissolved sulphides below the redoxcline.

Figure 31 also shows the typical distribution of chlorophyll in the lake; a more or less symmetrical curve of bacterial chlorophyll, peaking at very high concentrations near the redoxcline, and a zone above the

redoxcline containing both algal and bacterial chlorophyll.

The remote location of the lakes was a major disadvantage in the determination of chlorophyll concentrations. At first (to April 1981) samples were filtered in the field and the dried filters returned to the laboratory for extraction in 90% acetone. This proved to be unsatisfactory as some breakdown in bacteriochlorophyll occurred. In a personal communication Professor Norbert Pfennig agreed that air drying of filters results in destruction of bacteriochlorophylls, and suggested other procedures which could be tried to suit the particular circumstances of this study. These included the simultaneous extraction and conversion of bacteriochlorophylls in the field into the more stable bacteriophaeophytins (using methanol containing 0.1 mol/L citric acid), and the return of anaerobically closed water samples to the laboratory under cold dark conditions, followed by extraction in methanol, or a mixture of acetone: methanol of 7:2. It was decided to collect 30 mL samples as late in the field-trip as possible, keep them cold and dark on the return trip to the laboratory, and extract them as soon as possible in methanol. This was done first in May 1981 (Trip 4) so the results for January, March and April 1981 were determined using acetone extraction of filters dried in the field. Pigment breakdown on the filters was variable, but underestimates of bacteriochlorophyll of up to 30 per cent were measured when the two methods were compared.

During routine chlorophyll analysis absorption measurements were made at 850 nm, 750 nm and at the peak absorptions around 650-670 nm and 410-430 nm. No allowance was made in the spectrophotometric determinations for the presence of both Chlorophyll *a* and bacteriochlorophyll: in samples containing both chlorophylls peak absorptions only were measured. The red absorption peak in methanol of samples near the top of a close-interval profile could be as high as 665 nm, while that of samples below the redoxcline was usually 657 nm. Between the two, absorption peaks of intermediate value were recorded. Bacteriochlorophyll was predominant in most of the profiles, but in Sulphide Pool on 24 Feb. 1983 (Fig. 55) high concentrations of Chlorophyll *a* were also recorded, and this sampling has been chosen as the clearest example of the variation in peak absorption with depth. To a depth of 1.4 metres, the absorption peak was 665 nm, indicating the presence of Chlorophyll *a* only. The peak then declined to 659 nm within 0.20 metres, showing the presence of varying proportions of Chlorophyll *a* and bacteriochlorophyll, and from 1.6 metres was 657 nm, indicating the presence of bacteriochlorophyll only.

By mixing extracts of Chlorophyll *a* and bacteriochlorophyll it was found that there was a linear relationship between the red absorption maximum and the proportion of the two chlorophylls present (Table 4).

Absorption Peak. nm	Percentage Chlorophyll *a* present	Percentage bacteriochlorophyll present
665	100	0
663	67	33
661	50	50
659	33	67
657	0	100

Table 4. Red absorption peaks for different mixes of Chlorophyll *a* and bacteriochlorophyll.

The amount of Chlorophyll *a* and bacteriochlorophyll in any sample of intermediate peak absorption could therefore be calculated by

$$\text{Chl. } (\mu g/L) = X \times (\text{Abs.Max.} - \text{Abs.850}) \times C \times \frac{\text{mL solvent}}{\text{l. Filtered}}$$

where X = percentage of the particular chlorophyll present, based on the wavelength of the absorption peak,
and C = specific absorption coefficient (11.9 and 10.2 for Chlorophyll *a* and bacteriochlorophyll respectively).

The major bacteriochlorophyll present in the lakes was identified after the first field trip as Bacteriochlorophyll *d* (BChl*d*).* An acetone extract was scanned and the absorption peaks were compared with those given for the bacteriochlorophylls of green photosynthetic bacteria by Stanier and Smith (1960). The scan, with the peaks given by Stanier and Smith for BChl*d* marked on it, is shown in Figure 58 (Stanier and Smith actually called the pigment "Chlorobium 650", because of its absorption peak of 650 nm in ether. It was renamed Bacteriochlorophyll *d* by Jensen *et al.* (1964). Similarly "Chlorobium 660", the other major pigment of the green photosynthetic bacteria, became Bacteriochlorophyll *c*). The location and relative magnitude of the absorption peaks were in close agreement with those given by Stanier and Smith for BChl*d*. However, slight deviations were seen, an absorption maximum being recorded at 654 nm rather than the 653 nm of Stanier and Smith, and at 407 nm rather than 406 nm. When methanol was used the peak red absorption was consistently 657 nm, 2 nm lower than the value of 659 nm given by Stanier and Smith for this solvent.

* Confirming an earlier identification of it by Baker (unpublished, Tyler pers. comm.).

Given the techniques and instrumentation used in this study, and the known variability of chlorophyll absorption characteristics, these differences are not regarded as significant in the characterization of the photosynthetic bacteria present in the lakes, nor in their quantitative estimation via pigment extraction.

From May 1981 methanol was used in routine chlorophyll analyses and the absorption spectrum of BChl*d* in methanol is shown in Figure 59, together with that of Chlorophyll *a*. The red absorption peaks are readily separable, allowing the identification of mixtures of the two chlorophylls as detailed above.

In vivo scans were also made from time to time on samples from above and below the redoxcline in the two lakes. Cells were filtered onto cellulose nitrate filters, the filters were then air-dried, cleared with cedar oil, and scanned in a spectrophotometer. Figure 60 shows scans of whole cells made in this way for Lake Fidler on 6 April 1982. The absorption spectrum for the sample at 2.75 metres was typical of algal cells containing Chlorophyll *a*, with an absorption maximum at the blue end of the spectrum and a peak in the red at 665-670 nm. The spectrum has been added to the underwater light measurements in Figure 30 to show the relationship between the red *in vivo* absorption peak measured in the laboratory and the diminution of red light measured at the depth of the algal population in Sulphide Pool.

The sample taken at 3.0 metres, predominantly of green photosynthetic bacteria, gave a similar absorption spectrum, but the red peak was considerably higher at around 720 nm. Nonetheless, the red peak was lower than that usually recorded for *in vivo* scans of BChl*d* (725-740 nm, Gloe *et al.*, 1975).

All green bacteria contain BChl*a* to the extent of 5-10 per cent of the total chlorophyll content, and its presence is indicated by a minor absorption band (often seen as a shoulder only) at 809 nm in spectra of whole cells (Olson and Stanton, 1966). Such a shoulder can be seen in the photosynthetic bacterial scan in Figure 60. The subsidiary peak at 659-660 nm was due to breakdown of cells and extraction of BChl*d* by the cedar oil.

The spectrum at 3.0 metres has been added to Figure 29 to show the relationship between the *in vivo* spectrum of photosynthetic bacterial cells and the light present at depth in Lake Fidler.

Extremely high chlorophyll concentrations were observed during this study, Chlorophyll *a* values up to 1,190 µg/L and Bacteriochlorophyll *d* values up to 2,360 µg/L being measured. The maximum values at each sampling of Lake Fidler and Sulphide Pool are given in Table 5, together with a calculation of chlorophyll concentrations on an areal basis.

	Lake Fidler			
	Chl*a*		BChl*d*	
	µg/L	mg/m^2	µg/L	mg/m^2
31 I 81	32	7	2360	560
16 III 81	81	15	395	700
21 IV 81			1170	
28 V 81			1263	670
2 IX 81			805	450
17 X 81	43	5	1430	400
12 XII 81			1210	440
11 II 82			1170	320
6 IV 82	850	60	1730	680
	Sulphide Pool			
	Chl*a*		BChl*d*	
	µg/L	mg/m^2	µg/L	mg/m^2
2 11 81	1190	200	920	480
14 III 81	150	40	1709	310
24 IV 81	178	40	403	200
3 IX 81			439	225
18 X 81	194	20	1250	530
13 XII 81	1040	170	540	400
10 II 82			860	600
5 IV 82	281	50	800	440
5 XI 82			2240	440
24 II 83	290	90	1250	490

Table 5. Maximum chlorophyll values and concentrations on an areal basis for Lake Fidler and Sulphide Pool.

3.3.3 Details of individual profiles

The following is a detailed presentation, trip by trip, of the data collected by close-interval sampler across the redoxcline in the two lakes (Figs. 31-41, 44-53). Scales on the physicochemical diagrams are more or less constant from figure to figure, but those on the microbiological diagrams vary somewhat, as the data have been presented for ease of interpretation. The data shown in the microbiological figures are of the organisms found in the lakes on a regular basis. Other organisms were found from time to time; these are not included in the figures but details are given of their distribution in the text.

3.3.3.1 Lake Fidler

31 January 1981 (Fig. 31)

The redox drop on this occasion was abrupt, at 2.60 metres depth, with a fall of 300 mV within 0.10 metres. Redox potentials were more or less constant to 2.60 metres, though values from 2.40-2.60 metres were 30 mV less than those above, and more or less constant below 2.85 metres. Dissolved oxygen values were around 6.0 mg/L at the top of the profile but dropped sharply to 0.2 mg/L at 2.35 metres, and remained at this value to 2.55 metres, creating a distinct microaerophilic zone of 0.20-0.25 metres. Dissolved sulphides first appeared at 2.65 metres and increased evenly with depth to 34 mg/L at 3.2 metres. The dissolved oxygen and dissolved sulphides profiles did not overlap, as neither parameter was detected at 2.60 metres. Values for pH increased from 6.1 to 7.2 down the profile, most of the increase occurring from 2.45 metres, 0.15 metres above the redoxcline, to 2.80 metres, 0.20 metres below it. Bacteriochlorophyll *d* values were extremely high, with a maximum at 2360 µg/L immediately below the redoxcline. The bacteriochlorophyll maximum was very much a peak, as values dropped sharply above and below 2.65 metres. Nonetheless, high concentrations of bacteriochlorophyll were measured down to 3.20 metres, and also in the microaerophilic zone above the redoxcline. The latter finding was unexpected as it was presumed that the photosynthetic bacteria in the lake would be confined to the monimolimnion. In fact, Bacteriochlorophyll *d* was detected as high as 2.05 metres, some 0.60 metres above the redoxcline and the first detectable dissolved sulphides, in water containing 5.2 mg/L of dissolved oxygen. Chlorophyll *a* was detected in concentrations up to 30 µg/L to a depth of 2.50 metres.

The high levels of bacteriochlorophyll are readily explained when the counts of microorganisms are examined. Three of the "organisms" shown in Figure 31 contributed to the bacteriochlorophyll measured in the lake. Firstly, extremely high concentrations of single-celled *Chlorobium* were found, peaking at 39 x 10^6 cells/mL immediately below the redoxcline. These bacteria persisted to the bottom of the profile and were also counted as high as 2.25 metres, very high numbers being present in the microaerophilic zone. Secondly, large numbers (up to 37,000 filaments/mL) of the filamentous form of *Chlorobium* were seen, their distribution being similar to that of the single-celled forms, but with a broader peak immediately below the redoxcline. And thirdly, large numbers (up to 94,000 consortia/mL) of "*Chlorochromatium aggregatum*" were present at 2.30-2.50 metres. The consortium was seen at depths from 2.05 to 2.75 metres and was presumably responsible for much of the bacteriochlorophyll measured above 2.5 metres, and all that measured above 2.25 metres, where no cells of *Chlorobium* were observed.

The Chlorophyll *a* detected was due to two green flagellate algae. Firstly, a very sharply defined population of *Scourfieldia caeca* was observed at 2.30-2.50 metres. The maximum population of this minute bi-flagellate alga was 107,000 cells/mL, at 2.40 metres. Secondly, a population of the highly motile *Trachelomonas volvocina* occurred at much the same depths.

A fifth organism, unidentified but allied to the colourless bacterium *Achromatium*, was present in the profile. With numbers up to 16,000 cells/mL, the major part of its population was present from 2.35 to 2.55 metres, but it extended from 2.15 to 2.85 metres.

Four organisms counted in the profile are not included in Figure 31. The diatom *Rhizosolenia eriensis* was present from 2.0-3.2 metres; the highest concentration was at 2.0 metres (15,000 cells/mL), cell numbers declining rapidly with depth to 1000 cells/mL at 2.45 metres, and then persisting at 90-500 cells/mL to 3.2 metres. *Ankistrodesmus falcatus* var. *mirabilis* showed a similar distribution, but with a maximum count of 760 cells/mL at 2.0 metres, and less than 100 cells/mL below 2.30 metres. *Beggiatoa* was present in low numbers (up to 700 filaments/mL) below 2.4 metres. An unidentified bacterium was present from 2.15 to 2.85 metres, at concentrations less than 40,000 cells/mL, except at 2.40 metres where there was a distinct population peak of 150,000 cells/mL.

The organisms present in the profile showed a trimodal distribution of cells: *R. eriensis* and *A. falcatus* were at a maximum at the top of the profile; *T. volvocina, S. caeca, cf. Achromatium* and *"C. aggregatum"* and the unidentified bacterium were at a maximum at 2.30-2.55 metres; and *Chlorobium* was at a maximum immediately below the redoxcline.

In terms of biomass, as measured by extraction of pigments, the organisms in the profile containing bacteriochlorophyll vastly outweighed those containing Chlorophyll *a*. If cell volumes are used as a measure of biomass (see Table 6) the same observation is made, the maximum cell volume of *Chlorobium* outweighing by some 25 fold that of the next most significant organism. Cell volume calculations for the organisms above the redoxcline show them to be of equivalent biomass; at 2.40 metres the total cell volumes of *Trachelomonas, Scourfieldia, "Chlorochromatium", cf. Achromatium* and *Chlorobium* were approximately equal, at around 900 x 10^3 - 1,400 x 10^3 μm^3/mL.

Organism	Cell volume μm^3
Trachelomonas volvocina	2400
cf. *Achromatium*	100
Scourfieldia caeca	12
Beggiatoa	80
Single-celled *Chlorobium*	0.6
Filamentous *Chlorobium*	4
"Chlorochromatium aggregatum"	5*

Table 6. Cell volumes used in calculations of biomass by volume.
* Volume of photosynthetic component only.

16 March 1981 (Fig. 32)

The drop in redox potential was abrupt, with a fall of 310 mV within 0.10 metres. A distinct microaerophilic zone was again present, dissolved oxygen values below 2.70 metres being less than 0.05 mg/L. Dissolved oxygen values at the top of the profile, 0.50 metres above the redoxcline, were also low at around 1.0 mg/L. Dissolved sulphides were first detected at 3.0 metres, increasing with depth to around 32 mg/L at the bottom of the profile. Values of pH increased from 6.3 to 7.15 over 0.70 metres, most of this increase occurring across the microaerophilic zone. Large bacteriochlorophyll levels were again measured, peaking at 2,400 µg/L immediately below the redoxcline. The peak was much broader

than that seen on the previous trip, spanning some 0.30 metres and extending further into the monimolimnion. However, large amounts were again seen above the redoxcline, particularly in the microaerophilic zone, and a value of 50 µg/L was found for the highest sample in the profile, at 2.55 metres.

The large bacteriochlorophyll values, and their extension into the microaerophilic zone, were again due to *Chlorobium* and *"C. aggregatum"*. The counts of the filamentous form of *Chlorobium* again paralleled those of the single-celled form. The peak of *"C. aggregatum"* was significantly deeper in the profile than that seen in the previous sampling; on 31 Jan. 1981 the population of this consortium peaked 0.10-0.25 metres above the redoxcline, but on this occasion it peaked at the redoxcline, and far greater overlap occurred between *"C. aggregatum"* and *Chlorobium*. A very sharp population maximum of *S. caeca* was recorded at 2.90 metres, and this organism also peaked closer to the redoxcline (0.10 metres above against 0.20 metres on the previous trip). The population of cf. *Achromatium* was well defined; it increased in numbers from the top of the profile to a maximum of 55,000 cells/mL at 2.85 metres, then declined very suddenly, but persisted at reduced numbers a small distance beneath the redoxcline. Its location relative to the redoxcline was similar to that recorded on the previous trip. *T. volvocina* was present in the upper part of the profile, at numbers up to 1,000 cells/mL. *Beggiatoa* was present in much higher numbers, up to 4,500 filaments/mL, than previously. Its population peaked at 2.80-2.85 metres, immediately above the redoxcline. *R. eriensis* was present, again occurring throughout the profile, but instead of being in highest numbers at the top of the profile and declining with depth as on 31 Jan. 1981, numbers increased from around 4,000 cells/mL at the top of the profile to around 30,000 cells/mL at 3.10 metres and there was a broad population peak (of 12,000-30,000 cells/mL) at the top of the monimolimnion from 3.05 to 3.35 metres. These figures are only for cells with cell contents; many empty frustules were counted and their distribution more or less paralleled that of the cells with contents. It is not possible to determine the viability of this organism from cell counts, but it is likely that the distribution seen resulted from the decline of a population of this organism in the mixolimnion, and its subsequent settling out in the denser layers of the monimolimnion. Two other organisms were present in the profile; *A. falcatus* var. *mirabilis* was present throughout with numbers around 200/mL above the redoxcline and 50/mL below it, and *Cryptomonas* sp. was present above 3.0 metres, at 150-350 cells/mL.

In terms of biomass, those organisms containing Bacteriochlorophyll *d* again far outweighed those containing Chlorophyll *a*.

21 April 1981 (Fig. 33).

The redoxcline on this occasion was at 2.90-2.95 metres. There was a microaerophilic zone of 0.30 metres or so above the redoxcline, and no oxygen was detected below 2.90 metres. Dissolved sulphides were first detected at 3.0 metres, and increased with depth to 26 mg/L at 3.7 metres. Samples from 2.90-3.30 metres were analysed for chlorophyll, and only bacteriochlorophyll was found. Highest values were found immediately below the redoxcline, but the peak values were only around fifty per cent of those of the two previous sampling trips, and the profile of bacteriochlorophyll was more even in appearance.

Reduced numbers of photosynthetic bacteria coincided with the lower values recorded for bacteriochlorophyll. Numbers of single-celled *Chlorobium* were around seventy per cent of those recorded previously, but those of the filamentous form were very much reduced, only one sample below the redoxcline having more than 7,000 filaments/mL, compared with counts of more than 30,000/mL for the previous samplings. *T. volvocina*, *S. caeca*, *Beggiatoa* and *cf. Achromatium* showed similar distributions to those recorded previously. The distribution of *"C. aggregatum"* was interesting in that it was present in significant numbers throughout the profile, but had a population maximum of 44,000 consortia/mL at 2.65 metres, 0.20-0.25 metres above the redoxcline. *R. eriensis* was again present throughout the profile, but on this occasion with no population maximum, the organism being evenly spread at around 1,500 cells/mL. *A. falcatus* was also present throughout, but only at around 20 cells/mL. A large unidentified bacterium (Fig. 126) was present from 2.90-3.20 metres, with a distinct population maximum at 3.0 metres, 0.05 metres below the redoxcline (see Table 7). The location of this organism immediately below the redoxcline, and its precise layering (see particularly Lake Fidler, 2 Sept. 1981), suggest that it is an obligate anaerobe capable of maintaining a discrete position in the water column. It is probable that the organism is photosynthetic, but it has not been possible to confirm this as it was never viewed live in samples taken directly from the lake, and did not grow up in culture. Further work is required to better characterize this organism: in the meantime it is simply designated here as Bacterium A.

Depth m	Cells/mL x 10^3	
	21 Apr. 1981	28 May 1981
2.70		0
2.75		0
2.80	0	54
2.85	0	166
2.90	17	74
2.95	Sample lost	7
3.00	270	
3.05	81	
3.10	13	
3.15	27	
3.20	34	
3.25	0	
3.30	0	

Table 7. Counts of a large unidentified bacterium (Bacterium A - see text and Fig. 126) from Lake Fidler.

28 May 1981 (Fig. 34)

The redox profile on this occasion had less of a "zig-zag" appearance than those recorded previously. A drop in potential with depth was recorded from the top of the profile. However, values were similar at 2.80-2.90 metres, and a significant drop below this produced a definite redoxcline. Bacteriochlorophyll values peaked slightly higher than usual relative to the redoxcline. (The values for Eh and BChl *d* were taken separately and it is possible that an error occurred in the depth of sampling for one of the parameters. The counts of *Chlorobium* suggest that the redox sample was taken at the wrong depth, and the Eh profile should perhaps be raised by 0.05 metres or thereabouts). The shape of the bacteriochlorophyll profile, and the actual concentrations, resemble those of 21 April 1981. Large amounts of bacteriochlorophyll were also detected in the 0.30 metres above the redoxcline. Unfortunately, particularly in view of the different Eh profile, and the possibility of sampling for Eh at the wrong depth, no dissolved oxygen or sulphide determinations were made on this occasion.

The distribution of *Chlorobium* paralleled that of bacteriochlorophyll, significant numbers being present at the top of the profile. The distribution of *"C. aggregatum"* was similar to that of 21 April 1981; the consortium was present throughout the profile but peaked some 0.35 metres above the redoxcline. *R. eriensis* was present throughout in low numbers, with more cells above the redoxcline (200-1,000 cells/mL) than below it (60-350 cells/mL). The large unidentified bacterium seen in the lake on 21 April 1981

(Bacterium A) was again present, with a population maximum of 166 x 10^3 cells/mL (see Table 7) around 0.05 metres above the redoxcline.

2 September 1981 (Fig. 35)

The redoxcline on this occasion was at 3.05-3.10 metres, and was very noticeable due to more or less constant Eh values down to that depth. Dissolved oxygen values above the redoxcline were low; a maximum value of 1.05 mg/L was recorded at the top of the profile, and most values below this were 0.35 mg/L. A value of 0.35 mg/L was recorded at 3.10 metres, one sampling syringe below the redoxcline. As for previous trips, the samples for the various parameters were taken separately and some variation in depth of sampling may have occurred. Dissolved sulphides were first recorded at 3.15 metres. Values remained low to 3.30 metres and then increased more or less linearly with depth. Such a profile, with a distinct zone of low values immediately beneath the redoxcline, had not been recorded previously; dissolved sulphides usually increased linearly with depth (as they did on this occasion from 3.30 metres) from the level of the redoxcline. Bacteriochlorophyll was detected from 2.75-3.70 metres (no determinations were made above or below these depths) with maximum values of around 800 µg/L at the redoxcline. Significant concentrations were recorded above and below the redoxcline, and the profile did not show the pronounced peak of bacteriochlorophyll seen at the redoxcline on previous occasions.

Chlorobium was at a maximum immediately below the redoxcline, but the population was smaller than that seen previously. (Filaments of *Chlorobium* were present, but in low numbers (100 filaments/mL maximum) and have not been included in Figure 35). The increase in *Chlorobium* in the 0.15 metres above the redoxcline was abrupt. Very few cells were recorded above 2.95 metres and the bacteriochlorophyll measured at 2.75-2.90 metres was presumably due to *"C. aggregatum"*, which peaked sharply at 2.90 metres at 11,000 consortia/mL. *T. volvocina* peaked (at 800 cells/mL) 0.05 metres above this, and *S. caeca* peaked (at 28,000 cells/mL) 0.05 metres below. *S. caeca* was particularly sharply stratified on this occasion; only three sampling syringes contained appreciable numbers of the organism, and the decline from 2.95 to 3.00 metres was acute. cf. *Achronatium* was also precisely layered, 0.05-0.20 metres above the redoxcline, extending only slightly below the cline. *Beggiatoa* showed a similar distribution but extended more into the monimolimnion. *Cryptomonas* was present (at approx. 15 cells/mL) above the redoxcline. The large unidentified

Bacterium A occurred across the redoxcline, with a population maximum of 37,500 cells/mL at 3.05 metres (Table 8).

Depth m	Cells/mL
2.95	0
3.00	430
3.05	37500
3.10	800
3.15	135
3.20	0

Table 8. Counts of the large unidentified Bacterium A (see Fig. 126) in Lake Fidler, 2 Sept. 1981.

17 October 1981 (Fig. 37)

This was the first sampling for which the 1.15 metre long 5 x 24 sampler was used.

The Eh profile was unusual as a distinct drop occurred at 2.50-2.55 metres, 0.25 metres above the redoxcline. Values of pH varied from 6.0 to 7.1 through the profile; a marked increase occurred from 2.45-2.70 metres and there appeared to be a slight bulge (0.15 units) at the redoxcline. Unfortunately, pH was not determined on the samples at 2.60 and 2.65 metres. Dissolved oxygen values at the top of the profile were high. Values decreased with depth to zero at 2.75 metres but the microaerophilic zone, so obvious on some previous samplings, was not present. Nonetheless, dissolved oxygen values in the 0.30 metres above the redoxcline were less than 2 mg/L. Dissolved sulphides were first detected at 2.75 metres, and increased rapidly with depth. It is interesting that sulphides were detected, and oxygen was not, in the sampling syringe above the redoxcline. Bacteriochlorophyll peaked at 1,430 µg/L immediately below the redoxcline, but was present in significant amounts both above and below it. Chlorophyll *a* was detected, at up to 40 µg/L, from 2.45-2.60 metres.

As very few consortia of *"C. aggregatum"* were observed on this occasion, the two forms of *Chlorobium* were the only significant contributors to Bacteriochlorophyll. The population peaked immediately below the redoxcline, but significant numbers were present above it, and cells were observed up to a depth of 2.45 metres, where the dissolved oxygen concentration was 2.2 mg/L. At 2.55 metres, where the dissolved oxygen concentration was 2.0 mg/L, the concentration of single-celled *Chlorobium* was 1.05×10^6 cells/mL. Unfortunately, the samples at 2.60 and 2.65 metres

were lost. *cf. Achromatium* was present in largest concentrations in the 0.30 metres above the redoxcline, but extended up to 2.20 metres and down into the monimolimnion. The distribution of *Beggiatoa* was similar, but with a maximum of 18,300 filaments/mL at 2.70 metres. *S. caeca* and *T. volvocina* were similarly located but counts were not made on samples from 2.60 and 2.65 metres. *Cryptomonas* was present at up to 55 cells/mL to a depth of 2.50 metres. Cells of the Chrysophyte alga *Synura petersenii* were also present to this depth, at concentrations around 20 cells/mL.

12 December 1981 (Fig. 38)

The irregularities in the profile for this occasion suggest either that the sample was taken improperly, or that the sampling location had been disturbed prior to sampling. Nevertheless, the data have been included for discussion purposes.

The Eh profile was significantly stepped 0.15-0.20 metres above the usual location of the redoxcline, and the decrease in Eh across the cline itself was much less than usually seen. The dissolved oxygen profile showed a bulge at 2.85-2.90 metres, and values decreased to zero at 3.05 metres, the value at 3.20 metres most likely being incorrect. Dissolved sulphides were first recorded below the redoxcline, but the shape of the profile suggests a great deal of disturbance. The bacteriochlorophyll profile was also irregular but showed a maximum of 1,200 µg/L beneath the redoxcline.

Single-celled *Chlorobium* was observed in a 0.25 metres band immediately beneath the redoxcline, but was present in only three sample syringes about it. Filaments of *Chlorobium* were similarly distributed but there was a very large peak of 14,500 filaments/mL in the first syringe beneath the redoxcline. *S. caeca* and cf. *Achromatium* were distributed through the upper part of the profile and also extended below the redoxcline. *Beggiatoa* was more evenly distributed, while *T. volvocina* was present in greatest numbers (1,000 cells/mL) at the top of the profile.

As the sampling was so obviously disturbed for some reason the results for this date are treated with caution in any subsequent discussion.

11 February 1982 (Fig. 39)

The redoxcline on this occasion was very distinct. However, based on the observation of dissolved oxygen, dissolved sulphides and bacteriochlorophyll, it is possible that the Eh reading at 2.75 metres was

an error, perhaps due to premature discharge of a sampling syringe. (It was not a pH effect, as E_7 values gave a similarly shaped profile). Values of pH increased sharply in the 0.20 metres above the redoxcline, particularly between 2.65-2.70 metres, where an increase of 1.1 units was recorded. This increase coincided with an increase in the concentration of bacteriochlorophyll which was at a maximum of 1,200 µg/L at 2.70 metres. Dissolved oxygen was present at 5.9 mg/L at 2.45 metres, thereafter declining quickly to zero by 2.75 metres. Dissolved sulphides were first recorded at this depth, thereafter increasing more or less linearly to 46 mg/L at 3.25 metres.

The redoxcline was placed high in the profile on this occasion to allow for the counting of microbes well below it. Fortunately, the distribution of organisms above the redoxcline was sufficiently compressed to allow the counting of the whole microstratification.

Single-celled *Chlorobium* displayed its usual distribution, peaking at the redoxcline but extending appreciably above it. Beneath the population maximum cell numbers decreased quickly with depth, and numbers below 3.15 metres were only ten per cent or less of those at 2.75 metres. At 3.55 metres, 0.80 metres below the redoxcline, 0.7×10^6 cells were counted per mL. The filamentous form of *Chlorobium* also peaked at the redoxcline. Its extension into the waters above the redoxcline paralleled that of the single-celled forms (but note that no count was made at 2.65 metres), as did its extension below, except that very few filaments were observed below 3.0 metres. On this occasion cf. *Achromatium*, surprisingly, displayed a similar distribution to *Chlorobium*, except that few cells were observed below 3.30 metres. (The sample at 2.65 metres was lost). *Beggiatoa* peaked above the redoxcline, but was present in lower numbers (2,000 filaments/mL) to 3.55 metres. Likewise, *S. caeca* was in greatest numbers above the Eh shift, peaking within 0.10 metres of it, and cells were observed in low numbers down to 3.35 metres. *T. volvocina*, at numbers up to 260 cells/mL, was confined to the upper part of the profile. "*C. aggregatum*" was present in greatest numbers below the redoxcline, at 2.80 metres (69,000 consortia/mL). The consortium had not been observed to peak beneath the redoxcline since 16 May 1981; on other sampling trips it had peaked above the redoxcline, on one occasion (21 April 1981, Fig. 33) 0.20-0.25 metres above it. The peak at 2.80 metres was particularly sharp, as the population then declined from 69,000 to 6,000 consortia/mL within 0.05 metres. The consortium was observed as high as 2.60 metres, and persisted in low numbers (500-1,000 per mL) to 3.60 metres. *Cryptomonas* was present to a depth of 2.75 metres at around 100 cells/mL. *A. falcatus* var. *mirabilis* was also present, at

around 200 cells/mL, to 2.75 metres and thereafter at around 20 cells/mL. *Synedia ulna* was also present in significant numbers, declining from 600 cells/mL at 2.40 metres to 70 cells/mL at 2.6 metres, and persisting thereafter at around 10 cells/mL. The large unidentified Bacterium A (Fig. 126) was present at the redoxcline and peaked at 2.80 metres at 59,000 cells/mL (Table 9).

Depth m	Cells/mL
2.60	0
2.65	0
2.70	17000
2.75	0
2.80	59000
2.85	8500
2.90	0
2.95	0

Table 9 Cell counts of the large unidentified Bacterium A (Fig. 126) Lake Fidler 11 Feb. 1983.

The results for this sampling were included, together with those of 6 April 1982 below, in a paper on the microstratification in Lake Fidler and Sulphide Pool which was presented at the XXIInd Congress of the International Association of Limnology at Lyon, France in August 1983 (Croome and Tyler, 1984, Appendix IV).

6 April 1982 (Fig. 40)

This sampling was one of the most interesting made from Lake Fidler, and illustrates the usefulness of the 1.15 metre long replicate sampler. On this occasion it worked very well indeed; the sample was obviously undisturbed and only one sample of the 120 was lost, that for microbiological counts at 2.90 metres.

The Eh profile was particularly precise; values declined slowly with depth from the top of the profile to 3.1 metres, then dropped 335 mV within 0.05 metres to give a very distinct redoxcline, and thereafter again declined slowly with depth. Values of pH were constant at 6.15-6.20 units from 2.40-2.85 metres, then increased to 7.20 within 0.20 metres, increasing thereafter only slightly through the redoxcline and into the monimolimnion. Dissolved oxygen concentrations decreased from 4.2 mg/L at 2.45 metres to zero at 3.05 metres, 0.05-0.10 metres above the redoxcline. A microaerophilic zone, where oxygen values were around 1.0 mg/L, was present in the 0.40 metres above the redoxcline. Dissolved sulphides

were first detected at 3.1 metres (at 0.2 mg/L), 0.10 metres below the last detected oxygen. Dissolved sulphides increased linearly with depth to 28 mg/L at 3.55 metres. Chlorophyll determinations were made on samples from 2.75-3.50 metres. Both bacteriochlorophyll *d* and Chlorophyll *a* were present from 2.75-2.90 metres, Chlorophyll *a* being measured in greater concentration (850 µg/L at 2.85 metres) than Bacteriochlorophyll *d* for the first time. Below 2.90 metres Bacteriochlorophyll only was detected. The values peaked at 1,730 µg/L at 3.1 metres, the same depth at which dissolved sulphides were first detected. There was a more or less linear increase in bacteriochlorophyll from 2.75 metres to 3.10 metres, and a similar decrease to 3.50 metres. Large amounts of bacteriochlorophyll were present in oxygenated waters above the redoxcline; at 2.95 metres, where the oxygen value was 1.0 mg/L, a value of 760 µg/L was recorded, and at 3.00 metres, where the oxygen value was also 1.0 mg/L, the value for bacteriochlorophyll was 1,130 µg/L.

The distribution of single-celled *Chlorobium* was identical to the profile of Bacteriochlorophyll *d*, with a maximum of 23×10^6 cells/mL at 3.10 metres. The count at 2.95 metres, where the dissolved oxygen value was 1.0 mg/L, was 13×10^6 cells/mL. The distribution of the filamentous form of *Chlorobium* was similar except that the peak concentration was 0.05 metres lower and no filaments were observed beyond 2.90 metres. "*C. aggregatum*", the other organism contributing to bacteriochlorophyll, showed a marked peak of 365,000 consortia/mL at 3.15 metres. It was not observed above 2.95 metres, and was present in lower numbers (10,000 consortia/mL) beneath the redoxcline. cf. *Achromatium* was present from 2.65-3.55 metres, with greater cell numbers above the redoxcline, but also persisting in high numbers below it. *Beggiatoa* showed a similar distribution, but peaked somewhat higher in the profile, at 2.80 metres. The distinct peak of Chlorophyll *a* at 2.85 metres was due mainly to a population peak of *S. caeca*, which was present at 670,000 cells/mL. The population occupied a particularly narrow stratum: only two samples with high numbers were counted. The sample at 2.90 metres was the one lost but from the Chlorophyll *a* value at this depth it is probable the count would have been similar to that at 2.75 metres, giving the extremely dense population of *S. caeca* a depth of only 0.05-0.10 metres. *T. volvocina* was also present in this part of the profile but only in concentrations up to 55 cells/mL. *A. falcatus* var. *mirabilis* was observed throughout the profile, at approximately 100 cells/mL above the redoxcline and 30 cells/mL below it. The large unidentified Bacterium A seen on the previous trip (Fig. 126) was again present, with a depth range of 0.10 metres only, and a maximum concentration

of 127,000 cells/mL (Table 10) at 3.10 metres, the same depth as the peak of *C. limicola* and the first detected dissolved sulphides.

Depth	Cells/mL
2.95	0
3.00	0
3.05	21000
3.10	127000
3.15	0
3.20	0

Table 10 Cell counts of the large unidentified Bacterium A (Fig. 126). Lake Fidler 6 April 1982.

If cell volumes are used to estimate biomass (see Table 6) the dominant organism was *Chlorobium*, with a total cell volume of 13,750 $\mu m^3 x10^3/mL$ at 3.10 metres. However, the other organisms also displayed high total cell volumes at their respective population peaks; a value of 8,000 $\mu m^3 x10^3/mL$ was calculated for *S. caeca* at 2.85 metres, 5,300 for cf. *Achromatium* at 3.10 metres, 3,050 for *Beggiatoa* at 2.85 metres and 1,800 for "*C. aggregatum*" at 3.05 metres.

12 April 1983 (Fig. 41)

The results for this date are a good illustration of improper use of the 5x24 sampler. The sampler needs to be moved forwards slightly when the sampling depth is reached. Failure to do this results in a sampling of a disturbed profile as water is entrained by the sampler on its way down. Such a sampling occurred on 12 April 1983. The redoxcline was at 3.15-3.20 metres, Eh values dropping 450 mV within 0.15 metres. Dissolved oxygen values near the top of the profile were around 3 mg/L and decreased with depth. However, the decrease was not uniform and oxygen was detected 0.20 metres below the redoxcline in the presence of high levels of dissolved sulphides. The dissolved sulphides profile was disjointed, and the readings of electrical conductivity taken throughout the profile further confirmed that the sampling was made in a disturbed system.

The disturbance was also reflected in the counts of microorganisms. However, the basic distribution of the organisms was readily apparent, and the results have been included. The single-celled and filamentous forms of *Chlorobium* showed parallel distributions and peaked below the redoxcline. cf. *Achromatium* straddled the cline, but was present in greatest concentration in the 0.20 metres above it. *Beggiatoa* was distributed throughout the profile, with slightly higher numbers above the redoxcline. The

distribution of "*C. aggregatum*" was unusual in that few consortia were observed above the redoxcline, there was a marked population increase across the cline, and numbers increased right to the bottom of the profile. The population of *S. caeca* was very precisely layered, maximum population numbers being counted over a depth of only 0.05-0.10 metres. The maximum count of 550,000 cells/mL at 2.90 metres was very high, and rivalled the count of 670,000 cells/mL recorded at 2.85 metres on 6 April 1982. *R. eriensis* was present throughout the profile, declining from 4,000 cells/mL at 2.35 metres to around 1,000 cells/mL near the redoxcline, but increasing again to around 3,000 cells/mL at 3.25-3.50 metres. Many empty frustules of this organism were again observed.

3.3.3.2 Sulphide Pool

2 February 1981 (Fig. 44)

The redoxcline on this date was at 1.55 to 1.60 metres, and Eh values dropped 290 mV within 0.10 metres. Dissolved oxygen levels were less than 2 mg/L below 1.0 metres, and declined to zero at 1.55 metres. No measurements of dissolved sulphides were made. Values of pH fluctuated slightly down the profile but, in general, increased from 5.7 at 1.00 metres to 6.2 at 2.20 metres. Bacteriochlorophyll was present throughout, but was at a maximum of 920 µg/L at 1.50 metres, 0.05-0.10 metres above the redoxcline. Chlorophyll *a* was also present throughout, but highest values were recorded in the 0.10-0.30 metres above the redoxcline, with a maximum value of 1,200 µg/L at 1.40 metres.

The concentrations of bacteriochlorophyll agreed with cell counts of *Chlorobium*, except that the latter peaked (at 12.1×10^6 cells/mL) at 1.55 metres, 0.05 metres nearer the redoxcline, and showed a much more highly stratified population than might have been expected. The samples for the various parameters were taken separately, however, and this could explain such disparities. The high Chlorophyll *a* values were due to the presence of a dense population of *S. caeca*, which was present at 570,000 cells/mL at 1.40-1.45 metres. Again, there was disparity between the maximum Chlorophyll *a* values and the highest numbers of *S. caeca*. Two other organisms were present in the profile; an unidentified bacterium was present at up to 215,000 cells/mL to 1.50 metres, and up to 20,000 cells/mL thereafter, and an unidentified flagellate was present from 1.20-1.70 metres, with a population maximum of 95,000 cells/mL at 1.50 metres.

14 March 1981 (Fig. 45)

A marked stepping occurred in the Eh profile on this occasion with a drop in potential of 130 mV at 1.35-1.40 metres, a continued fall to 1.55 metres, a rise to 1.75 metres, and a sudden decline of 160 mV to 1.80 metres at the redoxcline "proper." Values of pH increased slightly down the profile. Dissolved oxygen levels at the top of the profile were around 4 mg/L but declined to less than 1 mg/L below 1.25 metres and persisted at very low levels to 1.60 metres, giving a distinct microaerophilic zone of 0.35 metres. Bacteriochlorophyll was detected throughout the profile, but showed a distinct maximum of 1,700 µg/L at the redoxcline. Chlorophyll *a* was detected from 1.45-1.95 metres at levels up to 150 µg/L.

The peak population of single-celled *Chlorobium* was 21.2×10^6 cells/mL and matched the bacteriochlorophyll maximum at 1.75 metres. Cells were observed in the microaerophilic zone above 1.60 metres but by far the bulk of the population occurred below this depth. The filamentous form of *Chlorobium* showed a parallel distribution. The Chlorophyll *a* present was principally due to the two highly motile flagellates, *S. caeca* and *Cryptomonas*, which were present from 1.25-1.80 metres. The population of *S. caeca* displayed an even profile with a peak of 130,000 cells/mL at 1.55 metres, 0.20-0.25 metres above the redoxcline, while that of *Cryptomonas* was uneven, with population peaks at both 1.40 and 1.65-1.75 metres, at up to 23,000 cells/mL. There was some disparity between the population distribution of these two flagellates and the levels of Chlorophyll *a*, but again the samples were taken separately. (For the same reason, no attempt is made to compare actual cell numbers with Chlorophyll *a* concentrations). *Euglena* sp. was present to 1.70 metres with highest numbers (450 cells/mL) at the top of the profile. *T. volvocina* was present to 1.45 metres in concentrations up to 50 cells/mL. *A. falcatus* was present throughout, at up to 100 cells/mL above the redoxcline, and 30 cells/mL below it.

24 April 1981 (Fig. 46)

On this occasion no marked stepping was apparent in the Eh profile. However, values did decrease slightly from 1.50 metres to the pronounced redoxcline at 1.80-1.85 metres where the Eh value dropped by 230 mV. A marked increase in pH was associated with the first drop in Eh at 1.50-1.55 metres, and the increase persisted to 2.20 metres. The pH increased by 1.5 units throughout the profile. Dissolved oxygen values were around 2 mg/L at 1.25-1.50 metres, but this declined to 0.2 mg/L within 0.05 metres

and persisted at low levels to 1.85 metres, again creating a microaerophilic zone of 0.30 metres. Bacteriochlorophyll values were low, increasing slightly with depth to a maximum of only 400 µg/L at 2.30 metres. Chlorophyll *a* was detected from 1.50-2.20 metres at levels up to 180 µg/L.

Counts of single-celled *Chlorobium* were only 3.9×10^6 cells/mL at the population maximum at the redoxcline. Highly stratified populations of *S. caeca* and *Cryptomonas* sp. were present, peaking at 43,500 cells/mL at 1.75 metres (0.05-0.10 metres above the redoxcline) and 19,200 cells/mL at 1.65 metres (0.15-0.20 metres above the redoxcline) respectively. *T. volvocina* was present to 1.85 metres in concentrations up to 45 cells/mL.

3 September 1981 (Fig. 47)

The redoxcline on this occasion was at 1.85-1.90 metres. It was not as distinct as on previous occasions, and Eh declined only 80 mV over 0.15 metres. Dissolved oxygen levels were low; the concentration from 1.30-1.70 metres was 0.35 mg/L and no oxygen was detected below 1.75 metres. The first dissolved sulphides were detected at 1.90 metres, and thereafter increased with depth to 11 mg/L at 2.20 metres. Bacteriochlorophyll levels were low, with a maximum of only 440 µg/L at 1.75 metres.

Counts of single-celled *Chlorobium* were also low, with a population maximum of only 2.2×10^6 cells/mL at 1.75 metres. The profile was much more sharply defined, however, than that of Bacteriochlorophyll *d*. *S. caeca* and *Cryptomonas* were present again, on this occasion both peaking at 1.35 metres, 0.50 metres above the redoxcline, at 107,000 and 13,200 cells/mL respectively. A small unidentified flagellate was present from 1.00-1.45 metres, peaking at 9,300 cells/mL at 1.20 metres.

18 October 1981 (Fig. 48)

This was the first occasion on which the 1.15 metres long 5x24 sampler was used in Sulphide Pool.

The redoxcline was at 1.85-1.90 metres, but the Eh profile was unusual in that an increase in Eh was measured below the cline. The difference between the Eh values at the top of the profile and those at the bottom was only 170 mV. Values of pH were around 5.5 units to 1.70 metres, increased to 6.05 over the 0.20 metres above the redoxcline, and thereafter increased only slightly. Values dropped by 0.2 units at 2.55-2.65 metres. Dissolved oxygen values were less than 1 mg/L, and declined to zero by 1.90 metres. The first dissolved sulphides were detected at 1.95 metres; concentrations increased with depth to 15.6 mg/L at 2.35 metres and thereafter remained

more or less constant. Bacteriochlorophyll was detected throughout, but peaked at 1,250 µg/L at the redoxcline. Chlorophyll *a* was detected at 1.50-1.60 metres at up to 200 µg/L.

The counts of single-celled *Chlorobium* matched the concentrations of Bacteriochlorophyll *d*. Cells were present at all depths, and the counts peaked at 8.3×10^6 cells/mL at the redoxcline. The filamentous form of *Chlorobium* peaked at the same depth but was not present in significant numbers beneath this. Many filaments were present, however, above the peak, up to 1.75 metres. (The value at 1.90 metres is taken to be an error, though the reason for it is not apparent). *S. caeca* dominated the upper part of the profile, at up to 78,000 cells/mL. The population declined quickly below 1.70 metres and few cells were seen below 2.05 metres. *Cryptomonas* was also present, with a maximum of 430 cells/mL at 1.60 metres.

13 December 1981 (Fig. 49)

On this occasion a distinct redoxcline was present between 1.85 and 1.95 metres, with Eh values falling 350 mV. Unfortunately, the sample at 1.90 metres was lost. Values of pH increased from 4.8 at 1.30 metres to 6.0 at 2.05 metres and thereafter remained steady. Dissolved oxygen values were less than 2 mg/L, declining to zero by 1.90 metres. Dissolved sulphides were first detected at 1.70 metres; values remained at around 0.6 mg/L to 1.85 metres, and thereafter increased to 5-10 mg/L. The first sample in which dissolved sulphides were detected was 0.15 metres above the redoxcline, where the dissolved oxygen concentration was 0.8 mg/L. Unfortunately, the samples at 1.60 and 1.65 metres were lost. (No sulphides were detected in the sample at 1.55 metres). The data for the other physicochemical parameters and for the counts of the microorganisms show that the sampling was true, i.e. dissolved sulphides and oxygen were not recorded at the same depths due to disturbance in the water column. This was the only occasion in either Lake Fidler or Sulphide Pool on which both sulphides and oxygen were measured at the one depth and there was no suspicion that the sampling had been made in a disturbed system. Bacteriochlorophyll was present throughout the profile, with maximum concentrations of around 500 µg/L in the region of the redoxcline. Chlorophyll *a* was present from 1.30-1.85 metres, peaking at 625 µg/L at 1.60 metres and 1,050 µg/L at 1.70 metres.

Single-celled *Chlorobium* was present throughout, with maximum concentrations of up to 4.7×10^6 cells/mL below the redoxcline. The filamentous form was also present throughout, but displayed a population

maximum from 1.70 metres, 0.15 metres above the redoxcline, to 2.10 metres. *S. caeca* was present in high numbers from 1.45 to 1.75 metres, with a maximum of 530,000 cells/mL at 1.60 metres. *Cryptomonas* was more precisely layered, peaking at 30,500 cells/mL at 1.70 metres. The depths of the population peaks of these two organisms corresponded, perhaps coincidentally, with the depths at which the two peaks of Chlorophyll *a* were recorded.

10 February 1982 (Figs. 50-52)

Two close-interval samples were taken by day on this occasion, one from the surface to 1.15 metres and one from 1.10 to 2.25 metres. The higher profile was taken for algal counts only, the lower for the usual physicochemical and microbiological determinations. The results for the latter are presented in Figure 50, while algal counts for the two profiles are combined in Figure 51.

The redoxcline was particularly marked (Fig. 50) as the Eh potential dropped 420 mV between 1.65 and 1.70 metres. (No further readings were taken). There was a more or less even increase of pH with depth from around 5.4 at 1.10 metres to 6.1 at 2.30 metres. Dissolved oxygen levels were less than 2 mg/L at 1.10 metres and thereafter declined. Dissolved sulphide levels were less than 10 mg/L. At the redoxcline there was an overlap of these two parameters, but electrical conductivity measurements showed there was disturbance at this depth and the values of dissolved oxygen and sulphides at 1.70 and 1.75 metres should be ignored. Bacteriochlorophyll was distributed more or less evenly with depth (no determinations were made above 1.25 metres), but Chlorophyll *a* was not detected in any quantity.

Single-celled *Chlorobium* was present from 1.10-2.25 metres, with a population maximum at 1.35 metres and another at 1.75 metres. The latter was expected, as it coincided with the depth of the redoxcline and the first appearance of dissolved sulphides, but the former was unexpected and was, at first, thought to be an error arising from confusion of *Chlorobium* with, perhaps, a heterotrophic bacterium. However, closer observation showed this was not so, and the high values of Bacteriochlorophyll *d* at equivalent depths confirmed the presence of photosynthetic bacteria. The filamentous form of *Chlorobium* displayed a similar distribution and occurred at up to 30,000 filaments/mL at depths near that of the higher single-celled *Chlorobium* peak. *S. caeca*, *Cryptomonas*, and the newly described Chrysophyte, *Mallomonopsis tasmanica* Croome and Tyler were present in highest numbers at the very top of the 1.10-2.25 metres profile.

Figure 51 shows the algal counts made from the surface to 2.25 metres. Distinct population maxima, of 240,000 and 47,000 cells/mL for *S. caeca* and *Cryptomonas* respectively, occurred at 1.10 metres, a bloom of the large *M. tasmanica* was present in the surface waters, with a stratified population maximum of around 1,500 cells/mL at 1.55-1.85 metres, and *T.volvocina* was present from the surface to 1.80 metres at concentrations up to 400 cells/mL.

5 April 1982 (Fig. 53)

The sampling on this occasion was much disturbed near the level of the redoxcline. The disturbance was obvious when the physicochemical profiles were examined; it was not clear at which depth the redoxcline occurred, and there was considerable distortion in the dissolved oxygen profile. Determination of electrical conductivities confirmed the disturbance. However, the data have been included as they do provide some valuable information.

Dissolved oxygen concentrations were high, up to 6.0-7.0 mg/L in the 0.25-0.75 metres above the redoxcline. Bacteriochlorophyll was present at all depths, with maximum values around the redoxcline. Chlorophyll *a* was present, from 1.75 to 2.05 metres, with a maximum concentration of 280 µg/L at 1.90 metres.

Counts of *Chlorobium* suggested that the redoxcline was at 2.05-2.10 metres, and hence that the Eh values at 2.15 and 2.20 metres were erroneous. The single-celled form was found throughout the profile but was present in greatest numbers below 2.00 metres. The filamentous form displayed a similar distribution, with a maximum concentration of 125,000 filaments/mL at 2.10 metres. *S. caeca* and *Cryptomonas* peaked exceptionally close to the redoxcline; *S. caeca* was present in significant numbers from 1.95 to 2.15 metres, peaking at 110,000 cells/mL at 2.05 metres, and *Cryptomonas* likewise, peaking at 27,500 cells/mL at 2.05-2.10 metres. The consortium *"Chlorochromatium aggregatum"* was recorded in Sulphide Pool for the first time on this date. It was present in low numbers (around 100 consortia/mL) to 2.00 metres and peaked at 3,500 consortia/mL at 2.10 metres. Concentrations of *"C. aggregatum"* were not assessed below 2.15 metres due to observational difficulties during counting. *T. volvocina* was present in low numbers (10-100 cells/mL) throughout the profile, as was *A.falcatus* (10 cells/mL).

5 November 1982 (Fig. 54)

The redoxcline was located at 2.10-2.15 metres and was very distinct, the Eh value dropping 360 mV within 0.05 metres. Dissolved oxygen values were less than 0.5 mg/L from 1.40 metres and oxygen was last detected at 2.15 metres. Dissolved sulphides were first detected at 2.25 metres. A sharp peak of bacteriochlorophyll of 2,240 µg/L was observed at 2.10 metres, but significant amounts may also have been present at 2.05 metres (this sample was lost). Appreciable concentrations of bacteriochlorophyll were present both above and below these depths.

Single-celled *Chlorobium* peaked at 2.05-2.15 metres but was present throughout. The filamentous form was also present throughout with a peak concentration of 1.8×10^6 filaments/mL at 1.95 metres. *"C. aggregatum"*, recorded in Sulphide Pool for the first time in April 1982, was present from 1.40-2.40 metres, with a population maximum of 580 consortia/mL at 1.60-1.65 metres. Low numbers and the difficulty in counting this consortium account for the ragged distribution recorded. *Cryptomonas* was present from 1.40-2.00 metres with a maximum concentration of 340 cells/mL at 1.50 metres. *Paraphysomonas caelifrica* Preisig and Hibberd 1982 was found from 1.40-2.30 metres with a maximum concentration of 470 cells/mL at 1.55 metres. A small unidentified flagellate was present at concentrations up to 1,400 cells/mL to 1.85 metres, at which depth it suddenly declined. *T. volvocina* was present at around 10 cells/mL above the redoxcline. *S. caeca*, so prevalent in previous samples, was also present above the redoxcline, but only in concentrations of around 50 cells/mL.

24 February 1983 (Fig. 55)

The redoxcline on this occasion was at 1.85-1.90 metres, but the change in potential across it was the lowest recorded for Sulphide Pool; a drop of 50 mV only was recorded. Bacteriochlorophyll was at a maximum of around 1,200 µg/L at the redoxcline but significant concentrations were also present above it. Chlorophyll *a* was detected at up to 300 µg/L from 1.0 to 1.6 metres, and overlapped the presence of bacteriochlorophyll by 0.20 metres.

The cell counts suggested some disturbance had occurred in the water column. *S. caeca* was present in a layer from 1.15-1.45 metres at up to 140,000 cells/mL. *"C. aggregatum"* displayed two population maxima; one of 5,250 consortia/mL at 1.60 metres and one of 5,000 consortia/mL at 1.90 metres. Both the single-celled and filamentous forms of *Chlorobium* were

present in highest numbers below the redoxcline, though the latter was also present in significant numbers up to 1.50 metres.

3.3.3.3 Horizontal heterogeneity of microorganisms

One of the original aims of the study documented here was to detail the seasonal variation of the various organisms present across the redoxcline in the two lakes. To this end, their horizontal heterogeneity was assessed on 11 February 1982 in Lake Fidler, by taking two samples approximately 10 metres apart at the main sampling buoy (see Fig. 8) and one approximately 60 metres away at the north-eastern end of the lake. The samples were taken from 2.30-3.45 metres. (The redoxcline was at 2.75-2.80 metres). The results are presented in Figures 61 and 62.

The population profiles of *S. caeca* in the three samples were identical, with maximum concentrations at 2.70 metres. However, there was considerable variation in numbers; samples A and C, taken 10 metres apart near the usual sampling buoy, showed peaks of 165,000 and 860,000 cells/mL respectively, while sample B, taken some 60 metres distant, showed a peak concentration of 470,000 cells/mL.

The three profiles of cf. *Achromatium* were similar, but again large variations were seen in the number of cells present. The greatest variation was between samples A and B, which had maximum cell concentrations of 51,000 and 460,000 cells/mL respectively.

The profiles of *Beggiatoa* were similar, and the variation in numbers was not as great. Nevertheless, the maximum value for sample A was only 30,000 filaments/mL, while that for sample C was 87,000.

T. volvocina showed similar profiles in samples B and C, with peak concentrations only 0.05 metres apart, but the population was much reduced in sample A and was at maximum concentrations some 0.05-0.25 metres higher.

The single-celled form of *Chlorobium* showed the closest agreement in cell numbers for the three samples, maximum counts being 17.1×10^6, 16.9×10^6 and 13.1×10^6 cells/mL for A, B and C respectively. However, there were important differences between the individual profiles. Firstly, the population in sample A peaked 0.15 metres above that of samples B and C, and secondly, the population maximum of B was much sharper than that of A and C.

For the filamentous form of *Chlorobium* the depth of maximum concentration again corresponded for samples B and C, but many more filaments were present at the maximum in sample C and the spread of the profile was much greater, many more filaments being observed above and below the population

maximum. The profile for sample A corresponded to that of the single-celled form of *Chlorobium* in being higher in the water column.

"C. aggregatum" showed highest numbers in sample B, where an extremely high peak concentration of 630,000 consortia/mL was found at 2.90 metres. Peak concentrations in samples A and C were less, at 68,000 and 260,000 consortia/mL respectively, and occurred 0.10 metres higher.

The three samplings showed there was considerable horizontal heterogeneity in the microbial populations in Lake Fidler on 11 February 1982. Heterogeneity was expected at the time of sampling, but the large differences in cell numbers between the three samplings were surprising, especially as two were taken only 10 metres apart. Some of the differences may arise from the fact that the distance between successive sampling syringes is 0.05 metres. Although 0.05 metres is an unusually close sampling interval, it is nontheless relatively large when compared with the thickness of the stratification of some of the microorganisms. However, this would explain only some of the differences seen.

Vertical differences were also apparent in the profiles. The differences observed for *T. volvocina* and *"C. aggregatum"* can be explained in terms of cell motility, as these organisms are highly motile, but those seen for *Chlorobium*, a non-motile organism presumably at maximum concentrations due to cell division in suitable environmental conditions, are difficult to explain.

In conclusion, the sampling of 11 February 1982 showed a marked horizontal heterogeneity in the microbial populations of Lake Fidler, the distribution of the individual organisms being very "patchy." Accordingly, no assessment is made herein of seasonal variations of the various organisms present in the lake, except in the most general terms. No replicate sampling was carried out in Sulphide Pool, but likewise, comments on seasonal variations are made only in general terms.

3.3.3.4 Diurnal samplings

Successful samplings were made at night on two occasions; in Lake Fidler on 16/17 October 1981 and in Sulphide Pool on 10 February 1982.

Lake Fidler 16/17 October 1981 (Figs. 36 and 37)

On this occasion the routine close-interval sampling of the lake was made at 1300 hrs on 17 October (Fig. 37). The night-time sampling was carried out at 2350 hrs on 16 October (Fig. 36).

Similar profiles were observed in the two samples for dissolved oxygen and sulphides, and for Eh, except that a step in the redox profile observed 0.20-0.25 metres above the redoxcline at the day sampling was not apparent at the night sampling.

The profiles for some of the microorganisms are incomplete as samples at 2.60 and 2.65 metres were lost at both the day and night-time samplings. *Chlorobium* was similarly distributed in the two samplings, but many more cells were present at the day sampling and the population peaked more obviously immediately below the redoxcline. (Through an oversight the filamentous form of *Chlorobium* was not counted at the night sampling). *S. caeca*, *Beggiatoa* and *T. volvocina* all displayed a similar distribution in the two samplings and were present in equivalent numbers. cf.*Achromatium* was similarly distributed day and night except for one high cell count at 2.55 metres at the day sampling.

The most significant differences in the distribution of organisms between the two samplings occurred for *Cryptomonas* and *Synura petersenii*. At the day-time sampling *Cryptomonas* was present at up to 55 cells/mL to a depth of 2.50 metres; at the night-time sampling it was present to 2.80 metres at numbers up to 265 cells/mL. Cells of *Synura petersenii* were observed in very low numbers only (less than 40/mL) at the day-time sampling, but at night were observed at up to 3,600/mL, and the organism showed a distinct stratification at 2.45-2.55 metres. This night-time sampling of Lake Fidler was the only occasion on which *S. petersenii* was observed in significant numbers in the area of the redoxcline.

In summary, the two samplings were similar, and many of the organisms displayed similar distributions at equivalent concentrations. However, the distribution and concentrations of *Cryptomonas* and *S. petersenii* were different, as many more cells were present at night, *S. petersenii* in particular showing a marked stratification. In view of the discussion of horizontal heterogeneity above, the similarity of the two samplings is surprising, and is taken to indicate that the basic array of microorganisms across the redoxcline is maintained through the night. The disparities seen in the distributions of *Cryptomonas* and *S. petersenii* may be due to diurnal movements of these organisms, but could also be due to the marked heterogeneity previously observed in the lake.

Sulphide Pool 10 February 1982 (Figs. 51 and 52)

On this occasion two close-interval profiles were taken at each sampling, covering the depths from the surface to 2.20 metres. One sampling was made at 0130 hrs on 10 February, and the other at 1300 hrs. The results are shown in Figures 51 and 52 for the day and night-time sampling respectively.

The sampling coincided with a bloom of *Mallomonopsis tasmanica*. At the day-time sampling (Fig. 51) *M. tasmanica* was present from the surface to 1.20 metres, but with a dense stratification of around 1,500 cells/mL at 1.55-1.90 metres. At night (Fig. 52) the organism was more evenly distributed, though numbers fluctuated with depth. Again, cell numbers decreased to zero at a depth of just over one metre.

The only other alga in significant numbers in the surface waters was *T. volvocina*, which occurred from the surface to 1.80 metres in concentrations up to 380 cells/mL in the day sample, but was present only from 1.10 to 2.00 metres in the night sample. Its peak concentration at night, 740 cells/mL at 1.50 metres, was very marked. (Fluctuations in the profiles of *T. volvocina* and *M. tasmanica* were due to the low number of cells counted. The size of the samples collected, and the counting technique, were designed for the organisms associated with the redoxcline, small organisms which occurred in high numbers. It was not possible to count the larger, less numerous organisms present in the water column with the same degree of accuracy).

S. caeca and *Cryptomonas* were present below 1.00 metres in both samples. At the day-time sampling they both peaked distinctly at 1.10 metres. At night the populations were just as marked, but *S. caeca* peaked at 1.30 metres and *Cryptomonas* at 1.45 metres, both somewhat closer to the redoxcline.

In summary, both samplings showed a stratification of organisms throughout the water column. A stratification of *M. tasmanica*, present at 1.55-1.90 metres at the day-time sampling, was not apparent at night. Stratifications of *S. caeca* and *Cryptomonas*, however, were equally apparent at night as they were during the day, though slight differences in depth were observed. *T. volvocina*, distributed throughout the water column at the day-time sampling, was present at 1.10-2.00 metres at night, with a large maximum at 1.50 metres.

3.4 THE ORGANISMS OF LAKE FIDLER AND SULPHIDE POOL

3.4.1 Introduction

Very little has been published on the organisms of Lake Fidler and Sulphide Pool. King and Tyler (1983a) reported for Lake Fidler that "the most frequent algae of the mixolimnion were *Rhizosolenia eriensis* var. *morsa* W. & G.S. West, *Mallomonas* sp., *Cryptomonas* sp., *Phacus* sp., *Trachelomonas* sp. and *Euglena acus* Eh. Several species of desmids and diatoms were also present, in small numbers." They also noted that *Cryptomonas* sp., large numbers of a minute flagellate, and a variety of bacteria occurred in the microbial plate about the chemocline. For Sulphide Pool they reported (King and Tyler, 1983) that the phytoplankton "was more plentiful in numbers, and more diverse than that of Lake Fidler. A variety of species of desmids and diatoms, as yet unidentified, occurred in the mixolimnion, together with *Euglena acus* Ehrenb. and *Mallomonopsis* sp.". And further, that "the micro-organisms of the bacterial plate were essentially the same as in Lake Fidler."

A list of phytoplankton collected during this study by plankton net (pore size 25 μm) and close-interval sampler, from both Lake Fidler and Sulphide Pool, is presented below in Section 3.4.2, and the organisms of the microbial stratifications are discussed in detail in Section 3.4.3.

3.4.2 Species present

Phytoplankton

A list of the phytoplankton species observed in Lake Fidler and Sulphide Pool from January 1981 to April 1983 is presented in Table 11. Some 40 species were observed in Lake Fidler, and 60 in Sulphide Pool. Members of the Chlorophyceae were most numerous in Sulphide Pool (37 species), and most of these were desmids. Many of these desmids seen in Sulphide Pool are of rare occurrence in Australia but, surprisingly, one area in which some of them occur is the tropical north of the continent (Ling, pers. comm.). In Lake Fidler members of the Chlorophyceae were again most numerous (20 species), but members of the Chrysophyceae were also significant (14 species). Many of the chrysophytes listed in Table 11 have not been seen previously in the southern hemisphere, and *Mallomonopsis tasmanica* is a new species described from Sulphide Pool (Croome and Tyler, 1983b, Appendix II). As a result of the number and diversity of the chrysophytes in Lake Fidler and Sulphide Pool, and the lack of previous investigations of this group in Australia, a preliminary survey of the chrysophytes from waters throughout Australia has been included herein (Section 3.6).

	Lake Fidler	Sulphide Pool
Cyanophyceae		
Aphanothece sp.		x
Microcystis sp.		x
Oscillatoria sp.	x	x
Euglenophyceae		
Euglena acus		x
" *oxyuris*		x
Phacus curvicauda		x
" *tortus*	x	
Trachelomonas volvocina	x	x
Dinophyceae		
Peridinium sp.	x	x
Cryptophyceae		
Cryptomonas sp.	x	x
Chrysophyceae		
Chromophysomonas trioralis	x	
Chrysosphaerella brevispina	x	
Dinobryon bavaricum	x	
" *sertularia*	x	x
Mallomonas adamas	x	
" *areolata*	x	
" *calceolus*	x	
" *lychenensis*		x
" *mangofera*	x	
" *morrisonensis*		x
" *splendens*	x	
Mallomonopsis tasmanica		x
Paraphysomonas caelifrica		x
" *vestita*	x	x
Synura curtispina	x	
" *petersenii*	x	x
" *spinosa*	x	
Tribonema sp.	x	
Baccillariophyceae		
Cyclotella stelligera	x	
Eunotia monodon		x
" *pectinalis*		x
Fragilaria ulna	x	
Frustulia rhomboides		x
Navicula radiosa		x
Pinnularia biceps		x
Rhizosolenia eriensis		x
Tabellaria floculosa	x	

cont.

	Lake Fidler	Sulphide Pool
Chlorophyceae		
Ankistrodesmus falcatus var. *mirabilis*	x	x
" *spiralis*	x	
Closterium aciculare		x
" *dianae*		x
" *pseudodianae*		x
" *striolatum*		x
" *subulatum*	x	x
Cosmarium amoenum forma		x
" *contractum*	x	x
" *cucumis*	x	
" *quadrifarium* var. *hexastichum*		x
" sp.		x
Desmidium aptogonum var. *tetragonum*		x
Dictyosphaerium sp.	x	
Docidium baculum		x
Euastrum ansatum var. *triporum*		x
" *sinuosum* var. *subjenneri*		x
Eudorina elegans	x	
Gonatozygon brebissonii		x
Hyalotheca dissiliens		x
Kirchneriella obesa	x	
Micrasterias decemdentata		x
" *denticulata*		x
Mougeotia/Debarya sp.	x	x
Nephrocytium agardhianum	x	
Netrium digitus		x
Oedogonium sp.	x	
Pleurotaenium ehrenbergii		x
Scourfieldia caeca	x	x
Sphaerocystis schroeteri	x	
Spirogyra sp.		x
Spondylosium pulchellum		x
Staurastrum brachiatum		x
" *cerastes* var. *pulchrum*		x
" *elegans*		x
" *longebrachiatum*		x
" " forma		x
" *playfairii*	x	
" *sagittarium*	x	x
" *sonthalianum*		x
" *sublaevispinum*		x
" sp.	x	
" sp.	x	
Staurodesmus extensus	x	x
" *mamillatus*	x	
" *mucronatus* var. *subtriangularis*		x
Tetmemorus brebissonii		x
" *laevis*		x
Xanthidium sp.	x	
Zygnema sp.		x

Table 11. Phytoplankton species recorded from Lake Fidler and Sulphide Pool - January 1981 to April 1983. (Most of the Chlorophyceae were identified by Dr. Hau U Ling, and most of the Bacillariophyceae by Dr. D.P. Thomas).

Zooplankton

The standing crop of zooplankton is low in both lakes: very low numbers of very few species were observed either in plankton tows or samples from depth. The calanoid copepod *Calamoecia tasmanica tasmanica* Smith was present, as were the rotifers *Keratella* spp., *Filinia* sp. and *Polyarthra* sp. "Phantom larvae" of the dipteran *Chaoborus* were observed occasionally in samples taken by the close-interval sampler. The possibility of a vertical diurnal migration of *Chaoborus* across the O_2/H_2S interface in Lake Fidler was noted by King & Tyler (1982a), and a detailed account of this migration is soon to be published (Baker *et al.*, in prep.).

3.4.3 The organisms of the microbial layers

This section concerns firstly the dominant organisms of the microbial stratifications in Lake Fidler and Sulphide Pool, their ecology, their physiology, and their distribution within the lakes; and secondly, those organisms which occur only occasionally in the stratifications, but are of particular interest.

3.4.3.1 *Chlorobium* Nadson 1906 (Figs. 65-76)

The order Rhodospirillales is defined as comprising those bacteria that contain bacteriochlorophylls (BChl's) and carry out an anoxygenic photosynthesis (Truper and Pfennig, 1978). The suborder Rhodospirillineae comprises those bacteria that contain BChl *a* or *b* and contains the families Rhodospirillaceae (purple and brown nonsulphur bacteria) and Chromatiaceae (purple sulphur bacteria). The photosynthetic pigments of these bacteria are located in intracytoplasmic membranes of different types which are continuous with the cytoplasmic membrane. The suborder Chlorobiineae comprises those phototrophic bacteria that contain BChl *c*, *d* or *e*, and contains the families Chlorobiaceae and Chloroflexaceae. The pigments are located in non-unit-membrane bound, lens-to-cigar-shaped structures, the chlorobium vesicles, that underlie the cytoplasmic membrane. BChl *a* is also present in the cells, usually in small amounts.

The family Chlorobiaceae comprises species of green photosynthetic bacteria in which reduced sulphur compounds (sulphide, sulphur, thiosulphate) serve as electron donors. All species are strictly anaerobic and obligately phototrophic. Elemental sulphur is formed extracellularly and further oxidized to sulphate. Some simple organic substances are assimilated in the presence of sulphide and carbon dioxide. The cells have rigid cell walls, divide by binary fission, and are non-motile.

Members of the Chlorobiaceae are commonly found in the upper layers of the sulphide-rich black mud in freshwater and estuarine environments, and in the upper layer of the sulphide-rich hypolimnion of stratified holomictic or meromictic lakes. The type genus *Chlorobium* comprises five species; the green *C. limicola* Nadson 1906 and *C. vibrioforme* Pelsh 1936 containing BChl *c* or *d*, their brown counterparts *C. phaeobacteroides* Pfennig 1968 and *C. phaeovibroides* Pfennig 1968 containing BChl *e*, and the green *C. chlorovibroides* Puchkova and Gorlenko 1982.

In Lake Fidler green photosynthetic bacteria are present in large numbers and give a distinctive green colouration to the waters at the redoxcline. Counts were made by filtering 1 mL of lake water, staining

the filters in a phenolic erythrosin solution, clearing them with cedar oil, and counting cells under the light microscope at 1250x using a planapochromatic objective lens. A photographic record was made of each count. As examples of such preparations, two samples from Lake Fidler are shown in Figures 65 and 66. Three forms of photosynthetic bacteria can be seen: *C. limicola*-like rods, *C. vibrioforme*-like curved cells, and a twisted form of many cells joined end to end. (The twisted form, being conspicuous and in relatively low numbers, was also counted beneath the inverted microscope).

Ultrathin sections of the cells were prepared and two are shown in Figs. 67 and 68. The most obvious feature of the organisms, particularly in Fig. 67, is the presence of numerous vesicles around the periphery of the cell. These "chlorobium vesicles" are diagnostic for the Chlorobiaceae. When the ultrathin sections were observed it was difficult to discern the three cell types observed by light microscopy. The short rods and curved cells were easy to identify, and were of similar internal structure, but it was not possible to identify with confidence the sections of the twisted form. However, a direct TEM observation of the twisted form is shown in Fig. 69. The cells appear similar to those of the other two forms, being slightly curved, and containing numerous large vesicles.

Enrichment and agar shake cultures were prepared using the medium of Pfennig as modified by Pfennig and Lippert (1966) and standard techniques (Van Niel, 1971). The enrichment cultures were dominated by short rods. A direct TEM observation and ultrathin sections of these rods are shown in Figs. 70, 71 and 72. The only successful agar shake cultures produced similar cells.

The short rods observed in Lake Fidler, and those that grew in culture, are identified as *C. limicola*. Unfortunately, neither the curved cells nor the twisted chains of cells grew in culture. However, the curved cells are taken to be *C. vibrioides* and the twisted form is presumed to be closely related. To identify the bacteria more precisely pure cultures would be required of all the major forms present. As this has not been possible to accomplish to date, the photosynthetic bacteria in Lake Fidler are referred to here simply as being of the *Chlorobium* type. In the presentation of cell counts, however, (Figs. 31-41) separate data are presented for the different forms, in that the rod-shaped and curved cells are presented as *Chlorobium* and the twisted form as .

The green photosynthetic bacteria of Lake Fidler are the single, most impressive feature of the lake. The arrival at the surface of the lake of a sulphide-laden, green-coloured water sample enthralls even the most experienced limnologist. During this study bacteriochlorophyll concentrations up to 2,400 µg/L and counts of *Chlorobium* up to $40x10^6$ cells/mL were recorded from the depth of the redoxcline.

Maximum cell concentrations of *Chlorobium* (Figs. 31-41) were usually found at the depth at which dissolved sulphides were first detected, within one or two sampling syringes of the redoxcline, and were usually quite distinct, as cell numbers were much lower above and below. Such a distribution can be seen in Figures 31 and 32 where the large cell counts of *Chlorobium* coincide precisely with the profiles of bacteriochlorophyll. From an appreciation of work carried out on similar environments in other parts of the world, the location of the maximum concentration of cells immediately below the redoxcline was expected. However, as members of the Chlorobiaceae are obligate anaerobes and require reducing conditions for photosynthesis, a finding which was not expected was the presence of significant concentrations of cells in the permanently oxygenated microaerophilic zone above the redoxcline: on 6 April 1982 (Fig. 40) $13x10^6$ cells/mL were present 0.15 metres above the redoxcline at a dissolved oxygen concentration of 1.0 mg/L; on 17 October 1981 (Fig. 37) $1.0x10^6$ cells/mL were present 0.20 metres above the redoxcline at a dissolved oxygen concentration of 2.0 mg/L; and it was not unusual to count significant numbers of *Chlorobium* up to 0.40 metres above the redoxcline. The cells above the redoxcline appeared viable and successful cultures were grown with inocula from these depths. There are two logical explanations for the unexpected finding of the cells in the microaerophilic zone in Lake Fidler. Firstly, the sampler used in this study allowed a more precise microbiological sampling about the redoxcline than that carried out in other lakes containing *Chlorobium*. Secondly, the cells above the redoxcline are "stragglers" lost from their optimum position in the water column due to inefficient depth regulation. (In a personal communication, Pfennig has written that this latter part of the explanation "appears to me correct").

Significantly different distribution profiles of *Chlorobium* were observed from time to time. On 21 April 1981, for instance (Fig. 33), the population extended significantly into the monimolimnion, on 28 May 1981 (Fig. 34) the overall distribution of cells was more even (perhaps a consequence of the large population present at the previous sampling), and on 12 December 1981 (Fig. 38) the population maximum was again very broad.

Significant numbers of the twisted filamentous form of *Chlorobium* were present on all but one sampling occasion. On most occasions the distribution was similar to that of the single-celled forms, peaking immediately below the redoxcline, and extending well into the microaerophilic zone (e.g. Figs. 31 and 32). On other occasions the distributions were dissimilar; on 21 April 1981 (Fig. 33) the filamentous form peaked sharply 0.20 metres below the peak of the single-celled forms, and on 12 December 1981 (Fig. 38) the filamentous form peaked immediately below the redoxcline while the single-celled forms exhibited a much broader population peak.

The maximum concentration of the filamentous form of *Chlorobium* varied from 100-140,000 filaments/mL. (The former figure was recorded on 2 September 1981 but not included in Fig. 35). In terms of cell volumes the filamentous form usually contributed less than 1 per cent of the total *Chlorobium* biomass. The exceptions to this were seen on 11 February 1982 (Fig. 39) when it contributed 2.5 per cent, on 6 April 1982 (Fig. 40) when it was present in the highest numbers seen during the study (up to 140,000 filaments/mL) and contributed 4.0 per cent, and on 12 April 1983 (Fig. 41) when the single-celled forms were at a maximum of only 6.6×10^6 cells/mL (the lowest maximum recorded in Lake Fidler) and the filamentous form contributed 5.0 per cent to the total *Chlorobium* biomass.

Sub-surface light intensities in Lake Fidler were determined on eight occasions (see Figs. 9-18, and Fig. 28), and light was found to penetrate to a depth of 3.0-3.1 metres. The amount of light present at the population maximum of *Chlorobium* was found to be less than 0.1 per cent of that incident at the lake surface. Thus very little light energy was available for photosynthesis. Furthermore, underwater spectrophotometric scans showed that the light which was present at these depths was of limited wavelength, as the waters of the lake selectively absorb light energy at shorter and longer wavelengths (Fig. 29). The population of *Chlorobium* "at best" photosynthesises at crepuscular levels of red light, and no light at all reaches the bacteria when more than 3.0-3.1 metres of water is present above them.

The assemblage of green photosynthetic bacteria present in Sulphide Pool is similar to that in Lake Fidler, the organisms again occurring in such large numbers as to colour the water green. The population is shown in Figure 73, and again three cell types are evident: rod-shaped cells, curved cells, and long chains of cells. In Sulphide Pool, however, the chains of slightly curved cells are not twisted as in Lake Fidler, but instead lie in the one plane, and the distinction between curved cells and chains of cells is often difficult to make. Ultrathin sections of the

cells are shown in Figs. 74, 75 and 76. In the presentation of cell counts (Figs. 44-55) the rod-shaped and curved cells are presented as *Chlorobium* and the chains of cells as C.

Cell numbers in Sulphide Pool (Figs. 44-55) were generally lower than those seen in Lake Fidler. The greatest concentration of *Chlorobium* recorded was 22×10^6 cells/mL (Fig. 45) and maximum values were usually less than 10×10^6 cells/mL. When population numbers were at their highest a distinct maximum was again apparent at the redoxcline (e.g. Figs. 44, 45), but when cell numbers were relatively low the population was more evenly dispersed through the water column (e.g. Figs. 48,49). Significant numbers of cells were again present in the constantly oxygenated micro-aerophilic zone above the redoxcline.

The filamentous form showed a similar distribution to that of the single cells. In terms of cell volume it made up less than 5 per cent of the population on half the sampling occasions, but on three occasions it constituted 20, 28 and 75 per cent of the population (Figs. 49, 47 and 54 respectively).

Light penetration in Sulphide Pool was determined on 6 occasions (Figs. 19-26 and Fig. 28), and light intensity at the population maximum of *Chlorobium* was always found to be less than 0.03 per cent of that at the water surface. This was so despite the fact that the redoxcline in Sulphide Pool is around one metre shallower than that in Lake Fidler, as the waters of Sulphide Pool are more highly coloured and absorb light more effectively, particularly at shorter wavelengths (Fig. 30).

3.4.3.2 *Scourfieldia caeca* (Korsh. 1916) Belcher & Swale 1963 (Figs. 77-94)

Syn. *Cardiomonas caeca* Korshikov 1916
Scourfieldia magnopyrenoidea Huber-Pestalozzi 1961.

The genus *Scourfieldia* West 1912 was established with *Scourfieldia complanata* West 1912 as the type species. Five species are now recognised, the other four being *S. cordiformis* Takeda 1916, *S. quadrata* Pascher 1927, *S. conica* Schiller 1954 and *S. caeca* (Korsh.) Belcher & Swale 1963.

The cells are small (4 to 10 μm), indented at the apex, strongly flattened, and heart shaped, elliptical or rectangular in surface view. Two long, slightly unequal flagella arise from the apex of the cell.

Scourfieldia caeca was first described by Korshikov (1916) as *Cardiomonas caeca*. The description was very precise, and the drawings of the organism were very detailed. Huber-Pestalozzi (1961) recognised the organism as a member of the genus *Scourfieldia*, and renamed it *Scourfieldia magnopyrenoidea* from the size of its conspicuous starch grain. However, such a change in the specific name was invalid under Article 55 of the International Code of Botanical Nomenclature 1955, and the organism was renamed *Scourfieldia caeca* by Belcher and Swale (1963).

(The genus *Cardiomonas* Korsh. 1916 should not be confused with the genus *Cardiomonas* Schiller 1954 established for an organism similar to *Scourfieldia*, but different mainly in that the plastid is replaced by endosymbiotic Cyanophyceae. As the name had already been used by Korshikov, Bourrelly (1966) renamed the genus *Pseudocardiomonas*).

Korshikov (1916) described the morphology, internal structure, and mode of division of naturally occurring cells of *S. caeca* in great detail. Belcher & Swale (1963) also reported observations of wild material. Belcher (1964) made further light microscope observations of the organism from culture, and Manton (1975) examined the microanatomy of *S. caeca* using electron microscopy, again using cells from culture. More recently the flagella structure of *S. caeca* from culture has been investigated in great detail by Melkonian and Preisig (1982). The observations reported below are from these five papers.

The cells of *S. caeca* are highly compressed, being round to heart shaped in surface view and cylindrical to ovoid in side view. Size varies from cell to cell; Korshikov gave an average length of 4 μm and width of 5 μm for cells in face view, while Belcher and Swale gave a width of 5-7 μm in face view, and of 1 μm in side view. The cells are naked; a ridge runs down each of the flat sides of the cell, but neither a cell wall nor a mucilage layer is present.

The anterior end of the cell is grooved to a greater or lesser extent, and from this apical groove extend two long and unequal flagella. The apical groove and the insertion of the flagella is at an angle of about 60° to the flattened plane of the cell. Korshikov gave an average length of the flagella as 22-25 μm and a difference in length between them of around 3 μm, while Belcher and Swale gave the lengths of the two flagella as 15-20 μm and 20-25 μm. The flagella are of the whiplash type, with a thin distal portion of 2-3 μm. A branched rhizoplast is present, one branch lodging

at the base of each of the two basal bodies of the flagella. The rhizoplast extends across the cell, anchoring near the middle of the inner side of the chloroplast.

A contractile vacuole is clearly visible on one side of the cell near the anterior end, but there is no eye-spot or pyrenoid. The nucleus is large, 1.5 μm in diameter, with a prominent nucleolus. The plastid comprises a massive keel-like base, and two anterior lobes, U-shaped in cross section, which extend to the extreme tip of the cell, so that for most of the cell the plasmalemma is underlain by a thick layer of thylakoids. The keel-like base of the plastid largely comprises a single (sometimes two) large crescent-shaped starch grain which appears to give shape and rigidity to the organism.

Cell division is longitudinal, the chromatophore and starch grain dividing in two. Two new flagella begin to appear before the cell has divided, the daughter cells each receiving one old and one new flagellum. The daughter cells are more rounded than the parent cell, but slowly acquire the shape of the latter.

Movement in *S. caeca* is unusual in that the cells travel backwards. The flagella beat in one plane only; under dark ground illumination they form a sharply defined line when the flat side of the cell is displayed, but when the cell is seen in narrow view the flagella only show up as a vague blur. The cell vibrates rapidly in the plane of the flagella, the posterior end, which travels first, describing an arc.

The cells of *S. caeca* differ from those of the type species, *S. complanata*, in being more flattened, producing a new pair of flagella before division, and regularly having one or two large starch grains present at their base.

S. caeca is similar to *Pseudoscourfieldia marina* (Throndsen 1969) Manton 1975. Originally named *Scourfieldia marina* by Throndsen (1969), the ultrastructure of *Pseudoscourfieldia marina* was investigated and compared with that of *S. caeca* by Manton (1975). Besides the presence of a pyrenoid in the marine organism, the most obvious difference between it and *S. caeca* was the possession of scales, both on the flagella and body. Manton (1975) renamed the organism *Pseudoscourfieldia marina* and allied it with the Prasinophyceae.

Taxonomically, *Scourfieldia* is presently regarded as a member of the Loxophyceae (Moestrup, 1982), or alternatively is placed in the Prasinophyceae (see Norris, 1980). Manton (1975) states that further work is needed before the true taxonomic position of *Scourfieldia* is known, and suggests this work should include some fine structure of the type species, namely *S. complanata*. (*S. caeca* is the only species on which there have been microanatomical observations). In the most recent discussion of the Prasinophytes, Norris (1980) includes *S. caeca* with other naked species in a group for which a distinct phyletic relationship with the scaly monads of the Prasinophyceae is uncertain.

The taxonomic position of the genus *Scourfieldia* is therefore not resolved and must await detailed investigation of fine structure of the various species, and, should it remain within the Prasinophyceae, clarification of the status and position of that taxon itself.

Ecological information on *S. caeca* is scant; Korshikov (1916) simply stated that the type material was found in the area of Kharkov in Russia; Belcher and Swale (1963) examined cells from ice covered water in the hole left by a fallen tree at the head of Elterwater in the English Lakes District; Belcher (1964) examined material from two temporary pools on Claife Height, Lancashire; and Melkonian and Preisig isolated cells from a small pond near Madingly (Cambridge, England).

Ecological information on the type species, *S. complanata*, is only a little more detailed. West (1912) reported that the organism was found by Mr. D.J. Scourfield in a pond on Leyton Flats, Essex. Mr. Scourfield reported that the organism "occurred in myriads, the water of the pond being distinctly green without any noticeable alteration in intensity from about the middle of January to the middle of April 1912. This period included one week of severe frost when the pond was covered with fairly thick ice. The green colour of the water was due entirely to this one organism." Scourfield himself reported *S. complanata* from three bomb craters in Epping Forest (Scourfield, 1944). Lund (1942) reported that *S. complanata* was often abundant from January to April in the Leg-of-Mutton Pond in Richmond Park, Surrey, where it occurred "exclusively above the bottom-deposit and not in the plankton."

In their paper on Lake Fidler, King and Tyler (1982a) stated that the upper levels of the plate of microorganisms across the chemocline were inhabited by "large numbers of a minute flagellate of bacterial proportions,

with long, apparently isokont flagella, probably of chlorophyte affinities." Prior to the study documented here the flagellate was known colloquially as "Matchbox", from its squat rectangular appearance when swimming. The location of "Matchbox" in the profile, and its identification, were given high priority at the beginning of this project, hence the detail with which this organism has been investigated, in particular by electron microscopy. It has been identified as *Scourfieldia caeca* and is shown in Figures 77-94.

The cells vary in size and shape. They are extremely compressed: in face view they are 3.0-5.0 μm long and 2.5-3.75 μm wide, and range from almost square, to almost round, to ovoid; in side view they are cylindrical to ovoid and around 1 μm wide.

Under the light microscope a distinct apical notch, from which two flagella arise, is visible on most cells. Under the scanning electron microscope this notch is seen to extend to a greater or lesser extent along one side of the cell (e.g. Fig. 81). Otherwise, given the methodology used to prepare the cells for observation, the outer surface of the naked cells appears smooth, except that one cell was seen with distinct grooves down its centre and around its circumference towards the posterior end (Fig. 82).

The flagella are long and unequal, with a fine hair-point (Figs. 77-80). Length of the longer flagellum ranges from 16-25 μm, and of the shorter from 10-23 μm. The difference in length of the two flagella is usually 5-6 μm; the maximum difference recorded was 7 μm, while one cell was seen in which the flagella were of equal length (23 μm). Several cells were observed with four flagella. Where this occurs there are usually two short and two long flagella; one cell, for example, had four flagella which were 15, 16, 20 and 22 μm long respectively. The hair-point extension of the flagella is usually around 3 μm long.

With phase contrast microscopy, a prominent crescent-shaped body is visible at the posterior of the cell, a large nucleus can be seen in the centre, and several smaller refractile granules are also visible.

Movement of the cell is backwards, with the flagella trailing. When the cell is in face view the flagella appear motionless, in side view they appear blurred. As the cell moves, the leading (posterior) end moves rapidly from side to side, giving the cell a box-shaped appearance.

Ultrathin sections of the organism are shown in Figures 83-94. Cells in true face or side view were rarely seen under the microscope, most sections being cut at various angles to the long and short-axis of the cell. Figure 83, however, is a section almost through the centre of the cell in face view, Figures 87 and 88 show the cell in side view, and Figure 84 is a cross section near the centre of the cell. No cell wall is apparent in any of the sections, and there are no scales, on either the cell or the flagella. The plasmalemma is smooth except for indentations apparent in some cells (e.g. Figs. 83, 84) corresponding to those observed by SEM.

The most obvious feature of the interior of the cell is perhaps the nucleus. It is relatively large and can easily be seen even under the light microscope; in Figure 87 it is particularly prominent, bridging the entire cell. The chloroplast is large, extending from a base at the posterior of the cell along the cell periphery to the anterior end (Fig. 83). In cross section it is seen to be bilobed (Fig. 84) with a small space only between the edges of the lobes. The only other area of the cell where the plasmalemma is not underlain by chloroplast is in the apical groove (Figs. 83 and 87). The chloroplast contains numerous thylakoids, thirty or so being observed in many cells. The single large granule at the base of the cell (sometimes two, see Fig. 85, presumably of a dividing cell), so obvious under the light microscope, is embedded in the chloroplast (e.g. Fig. 83). It is crescent-shaped and sometimes extends a considerable distance along the cell (Fig. 86). While its composition has not actually been determined, the grain is taken to be a large deposit of starch. Smaller refractile granules, also visible by light microscope, are present towards the centre of the cell, outside the chloroplast (Fig. 83). They appear opaque after the staining schedule used here, and are taken to be deposits of lipids or similar material. A contractile vacuole was observed in several cells adjacent to the nucleus (e.g. Fig. 86).

Figures 89-94 are serial sections through the apex of a cell illustrating the relationship between the cell, its apical groove, and insertion of the flagella. The apical groove, and the entry of the flagella, are at an angle of about 45^{o} to the flattened plase of the cell. In longitudinal section a rhizoplast is evident, extending across the cell towards the base of the chloroplast.

The microanatomy of the cell agrees broadly with the observations made on *S. caeca* from culture by Manton (1975). Two significant differences, however, are apparent. Firstly, the cells pictured by Manton had a very

narrow chloroplast, only five thylakoids wide, closely pressed against the plasmalemma. The chloroplasts of the cells pictured here are much larger, contain many more thylakoids, and take up a much greater proportion of the cell. Such a finding is not unexpected, as it has recently been shown that chloroplast structure in algal cells varies with the type of light used to grow the cells (Vesk and Jeffrey, 1977). One therefore might expect cells of *S. caeca* grown under standard culture conditions to possess a much smaller chloroplast than cells of the same organism collected from a darkly coloured water at extremely low levels of light.

The second significant difference between Manton's cells and those pictured here concerns the structure of the 'keel' present in the chloroplast. Earlier workers referred to a large keel of starch. After examining culture material, Manton emended the description of the keel, showing that starch was present only at its centre, most of the "keel" being made up of "relatively dense masses of semiopaque material interspersed with granules of unknown nature." The observations reported here, of naturally occurring cells, contradict the findings of Manton, and agree with the light microscope observations of earlier works. The "keel" in cells of *S. caeca* from Lake Fidler and Sulphide Pool is almost homogeneous and its appearance is consistent with the hypothesis that it is a single large starch grain.

The other granules reported here, presumably of lipid or similar material, were not present in the culture material examined by Manton. However, similar granules were seen by Korshikov (1916) in his original description of the organism.

The differences seen in the cell structure of *S. caeca* between cultured material, as reported by Manton, and naturally occurring material, as reported here, are interesting, particularly as one concerns the massive "keel" of starch, a diagnostic feature for *S. caeca*. They are of greatest significance, perhaps, in warning of the dangers inherent in studying the ultrastructure, and the ecology and physiology, of organisms outside their natural environment.

Scourfieldia caeca is a major component of the microbial array in Lake Fidler (Figs. 31-41). Maximum cell concentrations during this study ranged from 3,500-860,000 cells/mL, and contributed to Chlorophyll *a* values as high as 850 µg/L (Fig. 40). The organism was usually very precisely layered in the microaerophilic zone, by both night and day, and the population peak was most often present as a layer less than 0.05 metres deep

(e.g. Figs. 31-33). The distance of the population peak from the redoxcline varied; on several occasions it was 0.30 metres above it (e.g. Fig. 41), on others 0.10 metres above it (e.g. Fig. 32), and on others within 0.05 metres of it (e.g. Fig. 36). At these depths the amount of light present was always less than 0.5 per cent of that incident on the surface of the lake (see Figs. 9-18 and Fig. 28) and was mostly of wavelengths between 550-750 nm (Fig. 29).

In Sulphide Pool (Figs. 44-55) the very first sampling (Fig. 44) showed that *S. caeca* was a dominant organism in the microbial array about the redoxcline. Chlorophyll *a* values up to 1,200 µg/L were measured in the microaerophilic zone, and were due almost entirely to the presence of *S. caeca*. The population was often more dispersed than that seen in Lake Fidler (e.g. Fig. 45) but occasionally very precise population maxima were again present (e.g. Figs. 47 and 53). Its distribution was again confined to those depths above the redoxcline, maximum cell concentrations ranging from 0.10 metres above it (Fig. 46) to 0.60 metres above it (Figs. 50 and 51). At these depths the amount of light present was always less than one per cent of that incident on the lake surface (see Figs. 19-26 and Fig. 28) and was mostly of wavelengths between 600-750 nm (Fig. 30). The underwater spectrophotometric scans shown in Figure 30 were made on 2 February 1981 when a very large population of *S. caeca* was present, with maximum concentrations of 570,000 cells/mL at 1.40-1.45 metres (see Fig. 44). The effect of this population on the amount of light present at depth can be seen in the scans at 1.4-1.6 metres. In particular, there is a significant trough in the scan at 1.4 metres between 650-690 nm. The dotted line in Figure 30 is an *in vivo* scan of whole cells of *S. caeca* made in the laboratory. The absorption peak at the red end of the scan coincides with the dip in the scan at 1.4 metres depth in Sulphide Pool, and confirms that the large population of *S. caeca* present on this occasion selectively absorbed significant quantities of light at 1.4-1.6 metres.

One of the initial objectives of this study was the determination of seasonal fluctuations in the numbers of the various organisms present in the microstratifications of the lakes. However, the distribution of the organisms was found to be very patchy, and this applied particularly to *S. caeca*; a sampling in Lake Fidler on 11 February 1982 showed population differences of almost one order of magnitude between samples taken only 10 metres apart (Fig. 61). Consequently, the objective of detailing seasonal succession was abandoned and comments in this regard made herein are in general terms only. As might be expected, numbers of *S. caeca* in the lakes were found to be highest in the months December to April. However, significant

populations were present throughout the whole year, particularly in Sulphide Pool.

The population structure of *S. caeca* also leads to a questioning of the sampling interval used in this study. The sampler was constructed to take samples 0.05 metres apart. Small though it is, such an interval is clearly too great to assess properly the precise distribution of *S. caeca*, and perhaps consideration could be given to a modified version of the sampler so that samples could be collected at, say, 0.01 metre intervals. In this context it is interesting to note that the highest concentration of *S. caeca* in the two lakes (1.3×10^6 cells/mL in Sulphide Pool on 12 Dec. 1981) was recorded in a sample taken by the sampler pictured in Figure 6.

3.4.3.3 "*Chlorochromatium aggregatum*" Lauterborn 1906 (Figs. 95-109)

Syn. *Chloronium mirabile* Buder 1914.

Several bacterial consortia have been described in which a central, chemoorganotrophic, motile bacterium is covered by synchronously dividing green sulphur bacteria (Truper and Pfennig, 1978). The Chlorobiaceae are the only bacteria known to form such ectosymbiotic associations. The consortia were originally described as species. However, species names cannot be applied to symbiotic associations, and the names of these consortia have to be considered as not validly published, i.e. "without standing in nomenclature" (Truper and Pfennig, 1971). The names are still useful however, and are freely used in the literature; where they appear here they are placed in inverted commas.

The best example of such consortia is "*Chlorochromatium aggregatum*" Lauterborn, in which cells of green sulphur bacteria are regularly arranged around a large, colourless, polarly flagellated, rod-shaped bacterium of unknown physiological activity and taxonomic position (Stanier *et al.*, 1981). There is little doubt that the green bacteria belong to the genus *Chlorobium* (Truper and Pfennig, 1971). Growth and division of all cells occur synchronously, indicating a high degree of metabolic interdependence.

In his original description of "*C. aggregatum*", Lauterborn (1906) described it as being approximately elliptical, spindle, or barrel-shaped, narrowed and truncated at the ends, and 9-12 μm long by 5-7 μm broad. He was not aware of the central colourless bacterium but said the organism had a strong green coloured covering over a pale central part, the outer cells arranged end to end and forming a mantle. It was motile with the

help of a flagellum. Reproduction was by means of cross division, the "organism" extending in length and constricting in the centre.

Buder (1914), presumably unaware of Lauterborn's paper, described the consortium at great length and called it *Chloronium mirabile*. His paper included the first drawings of the consortium, as Lauterborn had not included illustrations in his original description. Buder's specimens were around 6 μm long and 2 μm wide.

Perfiliev (1914) added to the description of *"C. aggregatum"* by examining the green bacteria of the consortium. He found them to be 1.0-2.5 μm long and 0.6-0.8 μm broad, and allied them with *Pelodictyon* Lauterborn 1913. He also commented on the division of the consortium, stating that daughter consortia sometimes commenced division before they separated, so that four, and sometimes six, consortia were seen in a chain. He illustrated this, together with drawings of the consortia after various treatments.

Lauterborn (1916) repeated his original description of *"C. aggregatum"* and compared his observations with those of Buder.

More recently, Stanier *et al.* (1971) and Gorlenko *et al.* (1977) have included light micrographs of *"C. aggregatum"* in their respective texts, Caldwell and Tiedje (1975) have published electron micrographs of ultrathin sections of the consortium, and Pfennig (1980) has discussed it in a review article on syntrophic mixed cultures and symbiotic consortia. In the latter work, the hypothesis is presented that the central bacterium is either a sulphate- or sulphur-reducing bacterium. However, repeated attempts at culturing the central bacterium in isolation have failed. One possible reason for this is that the central bacterium of the consortium functions as an electron donor, and is obligately dependent on the green sulphur bacteria as electron acceptors: growth of the central bacterium in pure culture would therefore be impossible.

Several other consortia involving green sulphur bacteria are known. *"Chlorochromatium glebulum"* Skuja 1956, for instance, is similar to *"C. aggregatum"* but the cells of the green component contain gas vacuoles and are morphologically similar to cells of *Pelodictyon luteolum* (Schmidle) Pfennig and Truper 1971. Likewise, *"Pelochromatium roseum"* Lauterborn 1913 is similar to *"C. aggregatum"* except that the outer cells are not green but pinkish brown in colour. (Gorlenko *et al.* 1977 show an ultrathin section of this consortium). And *"Cylindrogloea bacterifera"* Perfiliev 1914 is a consortium consisting of a central filamentous nonmotile bacterium embedded

in a large slime capsule, which is covered by a layer of green bacteria, possibly *Chlorobium* or *Pelodictyon* species (Truper and Pfennig, 1971).

The consortia exhibit chemo- and photo-tactic responses and accumulate in optimum sulphide concentrations and light intensity (Pfennig, 1978). They have been found only in freshwater habitats, and may occur both in shallow pools above the mud and in the layers of green or purple sulphur bacteria in stratified lakes.

"Chlorochromatium aggregatum" occurs in both Lake Fidler and Sulphide Pool. The identification and investigation of this consortium, colloquially known as "Shape", posed many problems, and of the microbes present in the lake it was perhaps the most difficult to work with. However, with adaptation of techniques, particularly those involving SEM and the use of polylysine as an adhesive agent, the consortium was finally well characterised.

A light micrograph, SEM observations and ultrathin sections are shown in Figs. 95-109. When not in a stage of division (e.g. Fig. 97) the consortium is around 2 μm long and 1.5 μm wide, and the outer layer of photosynthetic bacteria are arranged more or less at right angles to the long axis of the consortium. The arrangement of the cells is variable however, as can be seen in Fig. 98, where a dividing consortium has cells irregularly orientated at one end and more or less longitudinally arranged at the other.

The outer layer, in which the cells are 1-2 μm long and around 0.5 μm in diameter, is only one cell thick. The dividing consortium, the stage most easily seen when observations are made by light microscopy, is commonly 3 μm long and 1.5-2.0 μm wide.

In their internal structure, the green photosynthetic bacteria of the outer layer are similar to those occurring singly in the *Chlorobium* populations of the lakes. The characteristic chlorobium vesicles are quite apparent, and the cells are identified as being of the *Chlorobium limicola* type.

The central bacterium is comparatively long, occasionally exceeding 3 μm in length, and of the order of 0.5-1.0 μm in diameter. The cell wall is often in close contact with those of the photosynthetic bacterial cells around it, and is covered with structures similar to those described by Caldwell and Tiedje (1975). These are hexagonal cups which presumably play a role in maintaining the shape of the consortium, and are evident in Figs. 101 and 104. The matrix of the central bacterium is lacking in

detail, but two large vesicles were observed near the cell wall in one section (Figs. 106 and 107). Such vesicles have not been reported previously in this bacterium, and their function is unknown.

It is the central bacterium which gives motility to the *"C. aggregatum"* via the beating of a flagellum. However, no flagellum was observed, either in motile, live material or in material preserved for cell counts or electron microscopy. Pfennig considers that the flagellum "should be there", and it "may be that it is shed off too readily" (Pfennig, pers. comm.).

Though patchy in its distribution (Fig. 62), *"C. aggregatum"* was found consistently in Lake Fidler, at maximum concentrations of 100-640,000 consortia/mL (Figs. 31-41). The population usually peaked in a layer 0.10 metres thick. The depth of this layer varied considerably in relation to that of the redoxcline; on one occasion the population maximum was 0.25 metres above the redoxcline (Fig. 33), on another 0.05 metres above it (Fig. 40), and on another 0.40 metres below it (Fig. 41). (The last example was confirmed by a sampling at night which has not been shown in the results). Thus, at any one sampling it was possible to find the population peak high in the microaerophilic zone, at or near the redoxcline, or well within the zone of dissolved sulphides. It is apparent that *"C. aggregatum"*, being motile, is able to take advantage of the varying conditions at depth within Lake Fidler, and moves through the water column in response to the environmental requirements of its two symbionts. The consortium undoubtedly contributes significantly to bacteriochlorophyll concentrations within the lake, but usually any particular bacteriochlorophyll value is derived from a mixture of *Chlorobium* and *"C. aggregatum"* in which *Chlorobium* is dominant. The exception to this occurred on 2 September 1981 (Fig. 35) when bacteriochlorophyll values of 175-430 µg/L at 2.75, 2.80 and 2.90 metres were due mainly to the presence of *"C. aggregatum"*. (This occasion also showed that the bacteriochlorophyll of the *Chlorobium* component of *"C. aggregatum"* was Bacteriochlorophyll *d*).

"C. aggregatum" was also observed in Sulphide Pool, but not before Sampling Trip 9, in April 1982; on 5 April 1982 (Fig. 53) a population maximum of 3,500 consortia/mL was present at or near the redoxcline, while on 5 November 1982 (Fig. 54) the consortium was present through most of the profile at concentrations up to 600 consortia/mL. While *"C. aggregatum"* was often difficult to observe under the inverted microscope, particularly in monimolimnetic waters, samples for algal counts were always examined closely, and it is unlikely that the consortium was present in Sulphide

Pool, at least in appreciable numbers, before April 1982. It is possible that the consortium was introduced to Sulphide Pool by way of sampling equipment previously used in Lake Fidler.

3.4.3.4 cf. *Achromatium* Schewiakoff 1893 (Figs. 110-119).

A large non-pigmented bacterium is always present in the microaerophilic zone of Lake Fidler. The cells vary in shape from almost spherical to long and oval, ranging in length from 3-10 μm. Light micrographs of the organism are shown in Figures 110-113, a drawing of a typical cell in Figure 115, and electron micrographs in Figures 114, 116-119. Freshly collected cells are motile, and revolve slowly around their long axis. No flagella are visible. Under the light microscope the most obvious feature of the cell is a number of large refractile spherules of regular appearance (Fig. 113). Around these are smaller bodies which have the appearance of sulphur granules. The cell wall is very difficult to see in freshly collected material but becomes quite visible as the cell loses its vitality.

The spherules are readily apparent when the organism is sectioned, and take up most of the volume of the cell (Figs. 116-119). The perimeter of these structures sometimes appears diffuse, but at other times is bounded by a definite membrane. Bodies corresponding to the sulphur granules observed in live cells were seldom seen in the sections, but the dense body present in Figure 117 is presumed to be one of these. Strange structures were seen in several cells, e.g. the structure adjacent to the vacuole in Figure 119, and the subsidiary vacuoles present at the site of division in Figures 116 and 118. In many sections the cell wall appeared to be covered with a fibrous mucilagenous material, e.g. Figures 116 and 118. In others, the outer edge of the cell was more distinct, but there was some evidence that the mucilage layer had been lost. No such mucilage was observed during SEM, e.g. Figure 114, but loss of such material during the preparation for SEM would be expected.

In its general morphology and appearance the organism is similar to *Achromatium* Schewiakoff, type genus of the Achromatatiaceae Massart, a family of uncertain affinity (Buchanan and Gibbons, 1974). Cells of *Achromatium* are spherical to ovoid or cylindrical, division is by constriction and they are motile by slow, jerky movements. The cells may contain sulphur and calcium carbonate inclusions. Photosynthetic pigments are absent. The organism is microaerophilic and apparently requires sulphides.

Two species of *Achromatium* have been described; *A. oxaliferum* and *A. volutans* (Buchanan and Gibbons, 1974). Cells of *A. oxaliferum* have a minimum width of the short axis of 5 μm and a maximum length of 100 μm. The cells usually contain small sulphur globules and much larger, highly refractile calcium carbonate crystals, but the latter may disappear under favourable environmental conditions. (They are possibly a device for pH control, De Boer *et al.*, 1971). Cells with calcium carbonate inclusions have a high specific gravity and therefore are found only on the bottom of pools and streams, usually in or on the mud. *A. oxaliferum* is found mostly in fresh water and brackish mud environments but has also been reported from marine muds (Nadson and Visloukh, 1923).

De Boer *et al.* (1971) examined *A. oxaliferum* by TEM and discovered peritrichously arranged appendages and a capsular slime. They advanced the hypothesis that the gliding motility of *Achromatium* is caused by peritrichous bacterial flagella beating in a slime layer that surrounds the cell.

The other species of *Achromatium, A. volutans,* has cells ranging in shape from spheres about 5 μm in diameter to ovals up to 40 μm long. They normally contain sulphur granules but lack internal calcium carbonate deposits. The organism is found in marine mud containing hydrogen sulphide and in decaying seaweeds.

The organism in Lake Fidler is also similar to *Macromonas* Utermohl and Koppe 1924, a sluggishly motile, colourless, cylindrical or bean-shaped organism (see La Riviere and Schmidt, 1981). *Macromonas* has a polar flagellum (perhaps a flagella tuft) and is characterized by the presence of several large refractile spherules, supposedly of $CaCO_3$ though proof of this is lacking. In addition, small sulphur globules may be present. Cells of *Macromonas* are often found in habitats where *Achromatium oxaliferum* also occurs.

As a definite identification of the organism present in Lake Fidler has not been possible, it is simply named here as cf. *Achromatium*. Although at the end of the size range reported for *Achromatium*, it has the same overall morphology, mode of division, and general appearance of this organism. Moreover, it appears to have a mucilagenous layer and mode of locomotion similar to that reported by De Boer *et al.* (1971) for *Achromatium*. *A. oxaliferum* usually has calcium carbonate granules instead of the large spherules seen here, but these may be absent in suitable environmental conditions, and are completely lacking in *A. volutans*.

Achromatium has never been cultured and its physiology remains unknown. From field observations, it is microaerophilic and apparently requires sulphides, occurring most frequently on mud substrates (La Riviere, in Buchanan and Gibbons, 1974). In Lake Fidler it does occur in the microaerophilic zone, but well above where sulphides are present, though the cells do appear to contain sulphur granules. In addition, some of its internal features, e.g. the vacuoles at the site of division, are not usually found in *Achromatium*.

Samples containing this organism, together with many of the figures shown here, have been sent to Dr. Karen Schmidt at Gollingen University in West Germany. She feels the organism may be related to *Achromatium*, but gives its location in the water column, small size, lack of calcium carbonate granules, shape after division (elliptical rather than spherical), and the differences in internal structure as reasons for regarding it as a different (unknown) taxon. Dr. Schmidt plans to show the organism to Dr. J. La Riviere of the Netherlands in an attempt to identify it more satisfactorily.

cf. *Achromatium* is a constant member of the microbial array in Lake Fidler (Figs. 31-41), and maximum concentrations during this study ranged from 16,000-140,000 cells/mL. The organism was found to be microaerophilic, with maximum cell numbers in the 0.40 metres above the redoxcline. It is motile and capable of maintaining a favourable position in the water column. When the organism was present in high numbers a distinct population maximum was observed; on 28 May 1981 this maximum was 0.30 metres above the redoxcline (Fig. 34), while on 2 September 1981 it was 0.15 metres above it (Fig. 34). Other population structures are exemplified by that of April 1981 (Fig. 33), when large numbers were present immediately above the redoxcline but over a greater depth, and 2 September 1981 (Fig. 35) when a similar but narrower population maximum was observed. It is incongruous that on the occasion of sampling Lake Fidler to assess the horizontal heterogeneity of organisms (Fig. 61) cf. *Achromatium* (which was found to be very patchy in its distribution) was at a maximum concentration in two of the samples at a depth below the redoxcline. It is possible that the depth of the redoxcline is plotted incorrectly, but it is not likely to be out by more than 0.10 metres. This places the population maximum "at least" at the redoxcline itself, still significantly lower than observed on other occasions.

cf. *Achromatium* was never observed in samples from Sulphide Pool.

3.4.3.5 *Beggiatoa* Trevison 1842 (Figs. 125 and 126)

Beggiatoa is the type genus of the bacterial family *Beggiatoaceae*. The organism is colourless, its cells are arranged in flexible chains, and it is mixotrophic or chemoorganotrophic, having a respiratory metabolism with molecular oxygen as terminal electron acceptor (Buchanan and Gibbons, 1974). It is aerobic or microaerophilic and deposits sulphur within the cells when grown in the presence of H_2S.

Six species of *Beggiatoa* are recognised at present; *B. alba*, *B. arachnoidea*, *B. gigantea*, *B. leptomitiformis*, *B. minima* and *B. mirabilis*. Buchanan and Gibbons (1974) list a further eighteen species or subspecies which have been described but for which acceptance depends on better characterization. They further comment that "although one might argue that all species are *incertae sedis*, it seems reasonable, if only for didactic purposes, to continue to recognise "these six species" largely because they may represent the generic extremes of size and, perhaps, of physiology." The six species range in size from filaments 1 μm or less in width, cell segments about 1 μm long (*B. minima*), to filaments 26-55 μm wide, cell segments 5-13 μm long (*B. gigantea*).

It is interesting that *Beggiatoa* is the organism on which Winogradsky founded his concept of lithotrophy (Winogradsky, 1888). Recently, however, several strains of *Beggiatoa* capable of growing autotrophically have been shown to grow better in the presence of low levels of acetate (e.g. Pringsheim, 1967). These particular strains, then, are not obligately chemolithotrophic, but rather mixotrophic in the concept of Rittenberg (1969) (Buchanan and Gibbons, 1974).

An organism akin to *Beggiatoa* is present consistently in the microstratification in Lake Fidler. The filaments are around 1 μm wide, and short to very long (up to 300 μm). Individual cells are around 2.5 μm long, and many contain refractile bodies taken to be sulphur granules.

In ultrathin section (Fig. 125) the cell wall, cross walls, site of the sulphur granules, and general matrix of the cell are all very similar in appearance to those seen in *Beggiatoa* by Morita and Stave (1963). (Unfortunately the only ultrathin sections of the organism obtained during this study were obtained early-on, before staining techniques were refined, and significant amounts of extraneous material is present in Fig. 125).

From its morphology, cell ultrastructure and ecology, the organism has been identified as *Beggiatoa*. From the size of its cells it is most closely related to *B. leptomitiformis*. However, given the doubtful status of the six species listed above, and of the eighteen other organisms awaiting better characterization, no attempt is made to ally it with any particular member of the genus. (It is significant perhaps that Morita and Stave also did not give a specific name to their organism, even though it was grown in culture and they examined its ultrastructure in great detail).

Beggiatoa is a constant member of the microbial microstratification in Lake Fidler (Figs. 31-41), and maximum concentrations during this study ranged from 700-38,000 filaments/mL. The organism was found in greatest numbers in the 0.40 metres above the redoxcline, but many filaments were also observed below the cline. As an example of this distribution, Figure 37 shows a maximum concentration of 18,300 filaments/mL at 2.70 metres, with a linear decline in numbers both above and below. Similar distributions can be seen in Figure 61, which illustrates the samplings made on 11 February 1982 to test for horizontal heterogeneity. The distributions in Figures 38 and 40 are more even, but again maximum concentrations occurred above the redoxcline.

Many of the filaments observed below the redoxcline appeared moribund, but as this was difficult to quantify the results displayed in Figures 31-41 and 61 are for total filament numbers. The organism is taken to be microaerophilic, with its optimum growth conditions occurring in the 0.40 metres above the redoxcline, and the filaments below the cline are taken to be those lost from the optimum growth conditions through inefficient depth regulation.

Beggiatoa was never observed in samples from Sulphide Pool.

3.4.3.6 *Trachelomonas volvocina* Ehrenb. 1883 (Figs. 120-122).

Members of the genus *Trachelomonas* are characterized by a rigid mucilagenous cell envelope, the lorica, which may be variously shaped and ornamented, and impregnated with iron, manganese and other elements (West *et al.*, 1980). The cells are motile, the emergent flagellum passing through an anterior pore in the lorica. Cytologically they resemble *Euglena*, and they are included in the Euglenophyceae.

The taxonomy of the genus is largely artificial, being based on the morphology and ornamentation of the lorica. These characters have been found to vary somewhat within the one species, depending for instance on the supply of iron and manganese (Pringsheim, 1953), and thus the present taxonomy of the genus is not completely satisfactory. Taxonomic schemes based on intracellular organization have been mooted, but as yet there is insufficient comparative ultrastructural data to establish satisfactory phylogenetic relationships within the genus (West *et al.*, 1980).

Leedale (1975) examined the fine structure of the envelopes in eight species of *Trachelomonas*. He found two distinct groups of species: those with envelopes constructed primarily of material with a finely granular texture, and those with 'weft' envelopes, constructed of flakes or fibrils in an amorphous matrix. *T. volvocina* was found to have a granular textured envelope of three layers, with a characteristic honeycomb construction of the middle layer. Leedale reported a similar lorica structure for a *Trachelomonas* he found blooming in a stone coffin in Adel churchyard, Leeds. He called this organism *Trachelomonas sp.* Wild (A) and suggested that it was closely related to *T. volvocina*. He was loath to call it *T. volvocina* as there were subtle ultrastructural differences in the patterning of the envelope layers, and a different internal organization to the cell. Most importantly, his *T. volvocina* had two large chloroplasts with inner pyrenoids, whereas *Trachelomonas sp.* (A) had a number of smaller discoid chloroplasts without pyrenoids.

The cell envelope of the organism reported here has the shape, collar formation, and ornamentation characteristic of *T. volvocina* (Fig. 120), and when sectioned shows the typical honeycomb pattern which Leedale illustrated for this species (Fig. 121). In its internal structure the organism is closer to Leedale's *Trachelomonas sp.* A, which he was loath to identify precisely as *T. volvocina*. In particular it has many small discoid chloroplasts rather than a few large ones (Fig. 122). However, since Leedale's observations, the chloroplast number and structure in algal cells have been shown to vary with the light regime under which the cells are grown (Jeffrey and Vesk, 1977; Vesk and Jeffrey, 1977). Given this, and the present taxonomy of the genus, with its emphasis on the shape and ornamentation of the cell envelope, the organism reported here has been identified as *T. volvocina*. Individual cells are spherical, 15 μm in diameter, and highly motile, with an emergent flagellum length of around 60 μm (Fig. 120). The cell envelope is a distinctive orange-brown in colour, and the organism is one of the first to catch the eye in any microscopical examination of the plankton.

Trachelomonas is a common component in many freshwater environments and the genus is well represented in lakes around the world. One particular occurrence of interest is in deoxygenated hypolimnetic waters during summer stagnation (Round, 1981).

T. volvocina is a regular member of the biota of Lake Fidler (Figs. 31-41), being most prevalent in the 0.75 metres above the redoxcline. Its population maximum varied during this study from less than 40 to 2,750 cells/mL, and occurred near the redoxcline, 0.30 metres above it, or up to 0.75 metres above it. Dissolved oxygen values at the population maximum were usually 1.0 mg/L or less, dropping on occasion to less than 0.1 mg/L. Light intensity at the depths of the population maxima were regularly less than 1 per cent of that recorded at the surface of the lake. At a day/night sampling in October 1981 (Figs. 36 and 37) the distribution of the organism at night was found to be similar to that observed during the day.

T. volvocina was also present in significant numbers on several occasions in Sulphide Pool (Figs. 44-55). Maximum cell counts were usually around 0.30 metres above the redoxcline, at oxygen levels less than 1 mg/L and light intensities less than 1 per cent of those measured at the surface. At a day-time sampling on 10 February 1982 (Fig. 51) the organism was found in significant numbers from the surface to 1.80 metres. A sampling on the previous night (Fig. 52) showed it to be in very low numbers from the surface to 1.0 metres, but present at 740 cells/mL (the highest count recorded in Sulphide Pool) at 1.50 metres, 0.15 metres above the redoxcline.

3.4.3.7 *Cryptomonas* Ehrenberg 1838

The systematics of the genus *Cryptomonas* are difficult, as there is a marked uniformity within it, and the organisms are difficult to fix and observe. Anton and Duthie (1981) have recently attempted to overcome the taxonomic problems by using cluster analysis statistics to determine the taxonomic value of certain characters and the affinities between the species. They found cell shape and size, number and position of the chloroplasts, structure of flagella and gullet system, and number and position of other included bodies to be of taxonomic significance. Using these characters they identified sixteen species of *Cryptomonas* from waters in Canada and the Northwest Territories.

A species of *Cryptomonas* occurs in Lake Fidler and Sulphide Pool. The cells are asymmetrical, as is typical of *Cryptomonas*, dorsiventrally flattened, and commonly 15 μm long and 9 μm wide. Given the features considered to be of taxonomic importance by Anton and Duthie, and the level of observation required to relate the organism found here with those found in Canada, no attempt has been made to identify the organism to specific level, and it is simply referred to here as *Cryptomonas* sp.

It is probable that *Cryptomonas* was overlooked in early studies on freshwater phytoplankton because of inappropriate techniques of collection and preservation (Anton and Duthie, 1981). Later studies demonstrated the importance of cryptophyte flagellates: Croome and Tyler (1973) found *Cryptomonas* to be a small but constant part of the standing crop of Lake Leake, a holomictic oligotrophic lake in eastern Tasmania; Munawar and Munawar (1975) found the Cryptophyceae to be important in the Laurentian Great Lakes; Round (1981) and Duthie (1979) have reported them to be significant in arctic and subarctic lakes respectively; Round (1981) has reported *Cryptomonas* as a dominant organism at depth in alpine lakes; and Ilmavirta (1984) has reported the Cryptophyceae to be important contributors to biomass in brown-water lakes in Finland.

There have been two reports of *Cryptomonas* at depth in lakes containing stratifications of photosynthetic bacteria. Firstly, Takahashi and Ichimura (1968) reported "an enormous population of photosynthetic flagellates (*Cryptomonas* spp.)....at the bottom of the thermocline" of Lake Haruna in Japan "near the base of the euphotic zone, and in contact with the H_2S zone. The total daily photosynthetic production by *Cryptomonas* was measured as 11 g C $m^{-2}day^{-1}$; this value would be near 5% of the total primary production of the lake." Lake Haruna is one of 10 Japanese lakes Takahashi and Ichimura have reported to have appreciable populations of photosynthetic bacteria at the top of their sulphuretted hypolimnia. Secondly, Steenbergen and Korthals (1982) have reported *Cryptomonas* at the top of the microbial array in Lake Vechten, where it exists under microaerophilic conditions at up to 100 cells/mL.

Cryptomonas was observed in Lake Fidler on five sampling occasions, and in Sulphide Pool on all but two sampling occasions. In Lake Fidler (Figs. 31-41), the organism was found to be a minor component of the microbial stratification about the redoxcline, with a maximum concentration recorded of 350 cells/mL on 16 March 1981, but in the more sheltered, pond-like Sulphide Pool (Figs. 44-55) it was found to be a major component of

the microbial array, and was recorded at up to 47,000 cells/mL.

On most occasions in Sulphide Pool (e.g. Figs. 46, 49, 53) *Cryptomonas* peaked sharply in high numbers 0.1-0.3 metres above the redoxcline, at very low oxygen concentrations (see particularly Fig. 46), and at light levels below 1 per cent of surface intensity. On other occasions the population was more evenly dispersed (e.g. Figs. 45, 54). In many ways the population distribution of *Cryptomonas* was found to be similar to that of *Scourfieldia caeca*; the two organisms exhibited a similar depth distribution (Figs. 45, 46 and 47), and sharp population peaks of the two were often very close together (Figs. 47, 51 and 53). In the diurnal sampling of Sulphide Pool on 10 February 1982 (Figs. 51 and 52) the population of *Cryptomonas* peaked sharply at both the day and night sampling; at 1300 hrs (Fig. 51) it peaked at 1.10 metres, the same depth at which the population of *S. caeca* was at a maximum, and at 0130 hrs (Fig. 52) it peaked at 1.45 metres, 0.15 metres below the population peak of *S. caeca*.

3.4.3.8 *Monosiga* Kent 1880 (Figs. 123 and 124)

The choanoflagellates are non-pigmented, heterotrophic microflagellates found in both fresh and salt water. They are of particular significance in the sea, where they form a major part of the bacteriovorous population. Occurring in densities of more than 1,000 cells/mL, the bacteriovores, principally choanoflagellates and colourless chysomonads, are capable of filtering 12-67 per cent of the water column per day, and play an important part in the "microbial loop," a recycling of materials by the microorganisms of the sea (Azan *et al.*, 1983).

The choanoflagellates are characterized by the presence of a collar of pseudopodia surrounding a central flagellum at the anterior of the cell. The organisms feed by catching food particles on the outside of the collar and engulfing them with pseudopodia arising from the peripheral base of the collar (Frenchel, 1982). They are solitary or colonial, and most have a lorica, which is often large and complex, surrounding the cell.

The genus *Monosiga* Kent 1880 comprises those species of choanoflagellates which are solitary and naked, i.e. not possessing a lorica. Five or six species of *Monosiga* are recognised, with size, shape of the cell, length of the flagellum, and length of the collar of pseudopodia being the diagnostic features (Bourrelly, 1968).

Choanoflagellates occur in low numbers from time to time in Sulphide Pool (Figs. 123 and 124). The cells are 2.5-5.0 μm long and 2.0-3.0 μm broad, with a central smooth flagellum around 15 μm in length, the distal portion being very fine, and a collar of approximately 20 pseudopodia. On the cells pictured in Figures 123 and 124 the pseudopodia are 8 and 3-4 μm long respectively. The cells have no stalk at the posterior end. In movement they display a slow rotation; the direction of movement is to the posterior.

It is possible that the organisms are juvenile stages of loricate choanoflagellates. However, no loricas or parts thereof were ever seen in Sulphide Pool, either associated with the cells or otherwise suspended in the water column. The organisms are taken therefore to be truly a-loricate and hence members of the genus *Monosiga*.

When compared with the described species of *Monosiga* the organisms in Sulphide Pool are most closely allied to *M. varians* Skuja. However, they are sufficiently different, particularly in the relative sizes of the collar, flagellum and cell, for there to be doubts concerning their exact alliance with *M. varians*, and they are designated here simply as *Monosiga sp.*

3.4.3.9 *Mallomonopsis tasmanica* Croome and Tyler 1983 (Figs. 200-204)

Mallomonopsis tasmanica is one of three new species of algae described as a result of this study, the other two being *Mallomonas plumosa* Croome and Tyler 1983, and *Mallomonas morrisonensis* Croome and Tyler 1983 (see Appendices I-III).

The cells of *M. tasmanica* are elongate, 3-4 times as long as broad (typically 35 μm x 10 μm), but distort when preserved in formalin or Lugol's solution and usually shed both scales and bristles. The scales are more or less orbicular and the bristles long and unequally forked at the tip. The size of the bristles is perhaps the most noticeable feature of this organism; bristle lengths of 38-140 μm were recorded, significantly greater than those previously reported for members of this genus (25 μm maximum) or, indeed, for the Chrysophyceae as a whole. A disrupted cell, scales, and bristles of *M. tasmanica* are shown in Figures 200-204. A complete description of the organism is included in Croome and Tyler (1983b) in Appendix II.

To date, *M. tasmanica* has been recorded only from its type locality, Sulphide Pool. For most of the time the organism is inconspicuous, but on 10 February 1982 it bloomed at concentrations of around 1,500 cells/mL. A check on phytoplankton net tows made by previous workers showed that blooms had occurred on two other occasions; on 23 Feb. 1977 and on 13 Dec. 1977.

The distribution of *M. tasmanica* at 1300 hrs on 10 Feb. 1982 is shown in Fig. 51. The organism was present from the surface to 1.2 metres, but was found in greatest numbers in a narrow band at about 0.6-0.9 metres, equidistant between the redoxcline and the lake surface. Water temperatures at these depths were 17-21^{o}C, dissolved oxygen levels were around 2 mg/L, pH was around 5.4, and the cells would have been receiving 2-5 per cent of surface PAR. No cells were observed below 1.2 metres, 0.5 metres above the redoxcline. A sampling at 0130 hrs on 10 Feb. 1982 (Fig. 52) showed a more even distribution of *M. tasmanica* but again no cells were observed below 1.30 metres.

From the above, *M. tasmanica* appears to be an opportunistic species which blooms from time to time in summer, and is able to select and maintain a favourable position in the water column.

Recent work in Finland has found Chrysophytes (and Cryptophytes) to be important contributors to biomass in brown-water lakes, their vertical migration being related to underwater radiation, and a possible "pick-up" of nutrients from hypolimnetic waters (Ilmavirta, 1984).

3.4.3.10 *Paraphysomonas caelifrica* Preisig and Hibberd 1982 (Figs. 205-201)

The genus *Paraphysomonas* comprises colourless, unicellular microflagellates (see later), its taxonomy being based on the structure of the silica scales which cover the cells. Some twenty five species are known, and the organism occurs in both freshwater and marine habitats (Preisig and Hibberd, 1982a,b).

Paraphysomonas is basically phagotrophic. The flagellum creates a current which moves water and bacteria towards the cell and along a ventral furrow. Particles which touch the furrow are phagocytized and, once ingested, pass along the cell, accumulating in posteriorally situated vacuoles where digestion takes place (Fenchel, 1982).

On 5 November 1982 an unknown species of *Paraphysomonas* was found in Sulphide Pool (Figs. 205-209). Preparations were made to publish a description of this 'new' organism, but a reeent description of it by Preisig and Hibberd (1982a) was, fortunately, discovered. They named the organism *Paraphysomonas caelifrica*, after the 'skyscraper' appearance of its spines. The cells occurring in Sulphide Pool had shorter spines than those of Preisig and Hibberd, but Preisig has also seen cells with short spines and regards the species as very polymorphic (Preisig, pers. comm.). Preisig was interested to know that the cells grew in large numbers in Sulphide Pool, as he has only been able to trace cells of this species after establishing enrichment cultures (pers. comm.).

P. caelifrica was present in Sulphide Pool on 5 November 1982 at concentrations up to 470 cells/mL, with a population maximum at 1.55 metres (Fig. 54). At this depth the temperature was 8.5^{o}C, dissolved oxygen 0.25 mg/L, and the cells would have been receiving around one per cent of surface PAR.

3.4.3.11 Other microorganisms (Figs. 126-132)

Many other microorganisms are observed from time to time in the microbial stratifications of Lake Fidler and Sulphide Pool. Several of these are pictured in Figures 126-132, together with the more common components of the microbial array. Most are insignificant in terms of biomass, occurring sporadically in low numbers. The exception is an unidentified bacterium, designated Bacterium A (see Fig. 126). This organism was present in high numbers on several occasions in Lake Fidler (see Section 3.3.3.1). Its distributions on these occasions were very discrete, as it was at maximum concentrations (up to 127,000 cells/mL) in a narrow band immediately below the redoxcline. It appears to be an obligate anaerobe, is capable of maintaining a discrete position in the water column, and may well be photosynthetic. However, it was never viewed when live, and did not grow up in culture. Further work is required to better characterize this organism: in the meantime it is simply designated here as Bacterium A.

3.5 OTHER OBSERVATIONS

3.5.1 Underwater transmissometry

A beam transmittance meter (transmissometer) was used on several occasions for *in situ* measurements of turbidity in both Lake Fidler and Sulphide Pool. Beam transmittance meters were used as early as the late 1930's for the resolution of the microstratification of particulate material in lakes, and for horizontal and vertical surveys that would otherwise have required a prohibitive number of samples (Ruttner and Sauberer, 1938; Whitney, 1938). But over the next 40 years there was remarkably little sustained use of transmissometry in lakes, possible deterrents being the relatively cumbersome nature of the apparatus, time-consuming measurements for fine resolution, and the unspecific nature of the measurements (Talling, 1981).

Recently, however, transmissometry was used to great effect in the measurement of seasonal changes in Esthwaite Water in the English Lakes District (Talling, 1981). The technique was used to monitor the vertical distribution of particulate material, mostly phytoplankton and Fe-compounds, during summer stratification over three years. It proved to be very useful, the chief merits being rapidity and reproducibility of operation, and high resolution.

The highly structured microstratification of organisms in Lake Fidler and Sulphide Pool suggested that measurements with a transmissometer might be useful in these lakes also, both in day-to-day sampling and in long-term monitoring. Consequently, the use of a Kahlisco beam transmittance meter No. 269WA150 was evaluated on three trips to the lakes. One profile from each lake is presented below.

Figure 63 shows Beam Attenuation against depth in Lake Fidler on 9 February 1982. (On any one occasion the measurements were made relative to 100 per cent transmission in air, and corrected against distilled water. Beam attenuation was calculated as

$$a = \ln T \times \frac{1}{x} \quad m^{-1}$$

where T = per cent transmission and x = light pathlength in metres). The profile showed low beam attenuation above and below a marked zone of high beam attenuation at 2.80-3.50 metres. No microbiological counts were made on 9 February but the depth of the zone of high beam attenuation matched precisely that of the organisms observed on 11 February (Fig. 16), once allowance was made for a variation in lake level of 0.18 metres. (The redoxcline is marked on both figures as a reference).

Figure 64 shows equivalent data for Sulphide Pool on 9 February. Again, the distribution of organisms in the area of the redoxcline was readily apparent and the profile was in close agreement with the cell counts (Fig. 51).

The transmissometer was heavy and difficult to use, being designed for oceanographic work carried out from large boats, and not limnological work carried out from small aluminium dinghies and rubber rafts. A further problem occurred with frequent fogging of the internal surface of the sensor lens: this required the dismantling and drying of the sensor, often in adverse weather conditions. With preserverance, however, meaningful results were obtained.

The most beneficial use of the instrument in Lake Fidler and Sulphide Pool is to locate and determine the density of the stratification of microorganisms about the redoxcline. However, these parameters are more easily determined by other techniques, and this, together with the cumbersome operation of the instrument and the non-specificity of its measurements, weighs against its use as a routine monitoring tool. It may be of some value in the assessment of horizontal homogeneity, but here a major drawback is that it is difficult to operate efficiently at the depth of the redoxcline. Significant movement of the underwater assembly can easily occur, and there is always a suspicion that the water column has been disturbed.

3.5.2 Inorganic carbon fixation

One of the aims of the work documented herein was to assess the use of radiocarbon for the determination of inorganic carbon fixation about the redoxcline. To this end, an experiment was carried out in Sulphide Pool on 12 December 1981, using samples from three depths.

Samples were collected from 1.8, 2.0 and 2.2 metres using the sampler pictured in Figure 6, and water from each depth was passed into 8 round 130 mL incubation flasks. (Opaque plastic tubing was used and all bottles were held within opaque covers). The bottles were then injected with 1 mL of 10 μC/mL of $NaHC^{14}O_3$, to give a concentration in the bottle of 7.7 μC/100 mL. One half of the bottles were further injected with 1 mL of a 1300μ mole solution of the Photosystem II inhibitor 3(3,4-dichlorophenyl)-1, 1-dimethyl urea (DCMU), giving a concentration in the bottle of 10μ mole, to allow differentiation between algal and bacterial photosynthesis. The opaque covers were removed from half the bottles injected with both reagents, and half those injected with C^{14} only, and the bottles

were lowered as quickly as possible to a depth of one metre. The remaining bottles were kept in their opaque covers in a light tight box in the shade.

The bottles were incubated from 1300 hrs to 1900 hrs, and the contents filtered in a field laboratory some 2-3 hours later. Counts were made using liquid scintillation techniques one week later. Carbon fixation was calculated after Vollenweider (1964). Alkalinity and pH were determined on the day of sampling, and samples were preserved for microbiological examination. The chemical and microbiological data are given in Table 12.

Depth	Cells/mL		C *	Total Alk.	pH
m	*Cryptomonas*	*S. caeca*		mmol/L	
1.8	24,300	750,000	1,500	0.14	4.55
2.0	53,000	1,320,000	8,150	0.42	5.0
2.2	25	0	400,000	0.68	5.3

Table 12. Cell counts, alkalinity and pH, Sulphide Pool, 12 December 1981.
*The distribution of the filamentous form of *Chlorobium* was taken to be representative of the photosynthetic bacterial population present.

The sample from 1.80 metres was dominated by algae, with very few photosynthetic bacteria present, that from 2.0 metres likewise but the bacterial numbers were larger, and that from 2.2 metres was dominated by photosynthetic bacteria. It was unfortunate that the experiment coincided with extraordinary low pH values in the lake; the pH at 1.8 metres was only 4.55 and that at 2.2 metres only 5.3. No accurate estimation of total CO_2 in the samples can be made from alkalinity values at these pH values: direct chemical methodology is required.

The scintillation counts are given in Table 13.

For a preliminary trial, the experiment worked well. The inoculum of C^{14} was of the correct magnitude; its addition had no effect on pH within the bottles and the maximum uptake of radiocarbon recorded was 15 per cent of that added.

Quite a difference was recorded between the sample replicates, and significant uptake occurred in all dark samples. Hence, there is some difficulty in the interpretation of results, particularly in those samples for which uptake was low. However, several observations can be made. At 1.8 metres significant photosynthetic uptake occurred in the light bottles untreated with DCMU and very little, if any, in the bottles treated with DCMU. This accords with the presence of large numbers of algae and few

photosynthetic bacteria. At 2.0 metres photosynthetic uptake in the untreated light bottles was even larger, and this accords with the larger numbers of algae counted in this sample. Significant photosynthetic uptake also occurred in the light bottles treated with DCMU, and this accords with the counts of photosynthetic bacteria made at this depth. At 2.2 metres photosynthetic uptake in the bottles treated with DCMU was greater than that in the untreated bottles, and this accords with the high numbers of photosynthetic bacteria counted. Uptake in the untreated light bottles still exceeded that of the untreated dark bottles however, and this accords with the presence of *Cryptomonas* in the sample.

		Counts/min./mL	
Depth m	Treatment	"Light" bottles	"Dark" bottles
1.8	Without DCMU	670,000	10,300
		575,000	12,200
	With DCMU	10,000	9,150
		10,100	7,200
2.0	Without DCMU	547,000	8,900
		1,170,000	13,300
	With DCMU	27,100	14,700
		23,500	10,700
2.2	Without DCMU	9,500	5,050
		17,500	5,550
	With DCMU	16,800	4,850
		23,400	6,350

Table 13. Scintillation counts (cpm/mL) of bottles incubated in inorganic carbon fixation experiment, Sulphide Pool, 12 Dec. 1981.

As explained above, it is not possible to use alkalinities to estimate the total CO_2 present in the water column at such low pH values. Accordingly, it is not possible to calculate accurately the uptake of radiocarbon in this experiment. However, it is possible to give figures for uptake of inorganic carbon which are known to be underestimates. Such figures have been calculated after Vollenweider (1964) using a pH_T factor of 1.0 (see Vollenweider, p.91). At 2.0 metres the photosynthetic fixation of inorganic carbon in the untreated light bottles is thus calculated at 228 µg C/L/hr, and in the light bottles treated with DCMU at 3.04 µg C/L/hr. The values are undoubtedly underestimates, and make the level of inorganic carbon fixation very significant indeed. The value for bacterial photosynthesis in the DCMU treated bottles can be compared with values given by Parkin and Brock (1980a, 1981) of around 6 and 15 µg C/L/hr in Knaack Lake, and by Gorlenko *et al.* (1973) of around 16 µg C/L/hr in Reproe Lake.

In summary, the experiment was a success in showing the possibility of using the C^{14} technique to assess carbon uptake in the lakes, and to differentiate between that taken up by algae and that by photosynthetic bacteria. Obvious modifications to the methodologies used in this trial are the use of a greater number of replicates, an accurate assessment of the total CO_2 present in the water, and more efficient sampling, inoculation, and return to the depth at which the sample is taken. (Inoculation of the samples *in situ* could also be explored). Uptake of organic molecules could also be assessed, by the use of labelled acetates for example. The radiocarbon technique is fraught with difficulties (e.g. Round, 1981), but appears to be worth persevering with in these lakes in order to assess the role played by the organisms stratified across the redoxcline. The preliminary observations documented above suggest that significant amounts of carbon are fixed by both the algae and the photosynthetic bacteria; further experimentation with both organic and inorganic radiocarbon would no doubt yield interesting and valuable results.

3.6 AUSTRALIAN CHRYSOPHYTES

3.6.1 Introduction

Many of the organisms found in Lake Fidler and Sulphide Pool are members of the Chrysophyceae *sensu* Lee (1980). As this algal class has received little attention within this country, a survey was made of water samples from many parts of the continent in an attempt to characterize the members of this taxon within Australia, and thus put the findings from Lake Fidler and Sulphide Pool in a national and global context. The survey has resulted in seven papers to date (see Appendices I-III, VII-X). Three new species have been described, and a further ten species recorded in the southern hemisphere for the first time.

3.6.2 Previous reports of Chrysophyceae in Australia

Some sixty taxonomic works on Australian freshwater algae have been published (for titles and area in which the studies were conducted see Ling and Tyler, 1984). The species of Chrysophytes reported in these papers, and their locations, are listed below. Only one author (Takahashi, 1978) examined the Chrysophyte material by electron microscopy.

Dinobryon bavaricum Imhof

New South Wales (Thomasson, 1973), and the Northern Territory (Ling and Tyler, 1984).

Dinobryon cylindricum Imhof ex Ahlstrom

New South Wales (Thomasson, 1973), Victoria (West, 1909), and South-eastern Queensland (McLeod, 1975).

Dinobryon divergens Imhof

New South Wales (Thomasson, 1973, Viyakornvilas, 1974), the Northern Territory (Ling and Tyler, 1984), South-eastern Queensland (McLeod, 1975), Victoria (West, 1909), and Tasmania (Cheng and Tyler, 1973).

Dinobryon elegantissimum West

Victoria (West, 1909).

Dinobryon elongatum Imhof

Victoria (West, 1909).

Dinobryon sertularia Ehren.

New South Wales (Playfair, 1912: 1915: 1921, Thomasson, 1973, Viyakornvilas, 1974), the Northern Territory (Ling and Tyler, 1974), and South-eastern Queensland (McLeod, 1975), and Tasmania (King and Tyler, 1981).

Mallomonas acaroides Perty

New South Wales (Playfair, 1921) and South-eastern Queensland (McLeod, 1975).

Mallomonas akrokomos Ruttner

New South Wales (Viyakornvilas, 1974), and Tasmania (Croome and Tyler, 1973).

Mallomonas australica Playfair

New South Wales (Playfair, 1921).

Mallomonas fastigata Zacharias

New South Wales (Thomasson, 1973).

Mallomonas litosema Stokes

New South Wales (Playfair, 1921).

Mallomonas papillosa Harris and Bradley

West Australia (Takahashi, 1978).

Mallomonas producta Ivanhoff

North-eastern Queensland (McLeod, 1975).

Mallomonas splendens (West) Playfair

New South Wales (Playfair, 1912: 1921, Thomasson, 1973, Viyakornvilas, 1974), Victoria (West, 1909), and the Northern Territory (Ling and Tyler, 1984).

Mallomonas striata Asmund

West Australia (Takahashi, 1978).

Mallomonas sp.

New South Wales (Viyakornvilas, 1974).

Mallomonas spp.

West Australia (Takahashi, 1978).

Paraphysomonas vestita (Stokes) de Saedeleer.

West Australia (Takahashi, 1978).

Synura australiensis Playfair, *Synura granulosa* Playfair, *Synura uvella* Ehren.

New South Wales (Playfair, 1915)

Synura echinulata Korshikov

The Northern Territory (Ling and Tyler, 1984). (Reported as a result of the study documented herein).

Synura spinosa Korshikov

New South Wales (Viyakornvilas, 1974), and Tasmania (Cheng and Tyler, 1973).

Synura petersenii Korshikov

The Northern Territory (Ling and Tyler, 1984).

McLeod (1975) also observed *Bicoeca petiolatum* (Stein) Pringsheim, *Chromulina ovalis* Klebs and *Ochromonas allorgei* Bourrelly in South-eastern Queensland.

Playfair (1921) observed *Bicoeca petiolatum* in New South Wales also, together with five species of *Chromulina, viz. flavicans* Ehr., *ochracea* Ehr., *ovalis* Klebs, *pyriformis* Playfair and *cuneata* Playfair.

3.6.3 Species observed during this study

The bulk of the Chrysophytes viewed during the study documented herein belong to the family Mallomonadaceae (see Preisig and Hibberd, 1982b). Members of the Mallomonadaceae have two unequal or subequal (but heteromorphic and heterodynamic) flagella, have plastids or are colourless, are solitary or colonial, and have a periplast covered with fine silica scales.

The taxonomy of the family has advanced greatly since the advent of electron microscopy, and because the main taxonomic features of the family are now the structure and arrangement of the scales covering the cells, electron microscope observations are obligatory in all taxonomic investigations.

Twenty seven members of the Mallomonadaceae were seen in samples from throughout Australia (see Fig. 133 for sample locations). The species are listed below. Several were observed from one locality only, and perhaps only one or two cells were seen, or even only one or two scales. The number of species found, and their distribution, will no doubt increase as more samples are viewed. However, many dozens of samples were examined during this survey, and the list below is a good indication of the presence and abundance of these organisms in Australia.

Three of the species are new and were described as a result of this study: *Mallomonas plumosa* (perhaps the most distinctive species of *Mallomonas* yet described), *Mallomonas morrisonensis*, and *Mallomonopsis tasmanica*. A further seven species are reported from the southern hemisphere for the first time: *Chrysosphaerella coronacircumspina*, *Mallomonas adamas*, *M. lychenensis*, *M. pseudocratis*, *Mallomonopsis elliptica*, *Paraphysomonas caelifrica* and *Synura echinulata*. (Two of these species, *Mallomonas lychenensis* and *M. pseudocratis*, have also been seen in South America, but are yet to be reported as occurring there - Durrschmidt, pers. comm.). Of these seven species, one, *Mallomonas adamas*, is reported for the first time since its original description from England, one, *Paraphysomonas caelifrica*, has previously been reported only from Europe, one, *Chrysosphaerella coronacircumspina*, is of widespread but limited distribution, and the remaining five are considered to be cosmopolitan. Of the sixteen species previously reported from the southern hemisphere (see list below), all but two (*M. mangofera* and *M. favosa*) are considered to be cosmopolitan.

Chromophysomonas trioralis (Takahashi) Preisig and Hibberd. (Figs. 134-137)
Takahashi 1973, Bot. Mag. Tokyo, 86, p.78, figs. 16-18.

Syn *Spiniferomonas trioralis* Takahashi 1973.

Observations: Lakes Fidler and Morrison in South-west Tasmania (4 April 1982 + 4 November 1982, and 28 May 1976 resp.), and Tooms Lake in eastern Tasmania, 29 May 1982, 13 June 1982.

Comments: Previously recorded from Europe (e.g. Kristiansen, 1980), North America (e.g. Stoermer and Sicko-Goad, 1977), South America

Durrschmidt, 1980), and Japan and South Africa (Takahashi, 1973). For habitat descriptions in Tasmania see King and Tyler (1982a: 1983) and Croome and Tyler (1972).

Chrysosphaerella coronacircumspina Wujek and Krist. (Figs. 138-139). Wujek and Kristiansen, 1977 in Wujek *et al.*, Mich. Bot. 16, p.191-193, figs. 4-7.

Observations: Lake Fidler (4 April 1982) and Lake Morrison (28 May 1976), South-west Tasmania.

Comments: Previously reported from Sweden, Alberta, and Michigan (see Wujek *et al.*, 1977). For habitat descrpitions in Tasmania see King and Tyler (1982a: 1983).

Crysosphaerella brevispina Korsh. 1942 em. Harris and Bradley. (Figs. 140-142). Harris and Bradley 1958, J. gen. Microbiol., 18, p. 75, figs. 6-9; pl. 2. Figs. 9-13.

Observations: Lake Morrison in South-west Tasmania, 28 May 1976.

Comments: Previously reported from Europe, North and South America, and Japan (see Durrschmidt, 1980). For habitat description in Tasmania see King and Tyler (1983).

Mallomonas adamas Harris and Bradley. (Figs. 143-144).
Harris and Bradley 1960, J. gen. Microbiol., 22. p. 768-769k figs. 27-29, pl. 6, Figs. 42-44 and 50.

Observations: Lake Fidler, South-west Tasmania, 4 April 1982, 12 April 1983.

Comments: Only two cells seen. Not reported in the literature since its original description from small water bodies in England, but has also been observed in Malaysia (Durrschmidt, pers. comm.). For habitat description in Tasmania see King and Tyler (1982a).

Mallomonas akrokomos Ruttner (Figs. 145-149).
Ruttner 1913 in Pascher, Susswasserflora Deutschlands 2, p. 36, fig. 52a,b.

Observations: Lake Leake in Tasmania, August 1971, Gumeracha Weir on the Torrens River in South Australia, 28 May 1982.

Comments: A distinctive species, easily recognized under the light microscope. Previously reported from Europe (e.g. Kristiansen, 1980), North America (e.g. Nicholls, 1982), South America (Durrschmidt, 1982), Japan (Takahashi, 1975), and Australia (by light microscopy) (Viyakornvilas, 1974). Gumeracha Weir is a mesotrophic environment; Lake Leake is oligotrophic (see Croome and Tyler, 1972).

Mallomonas areolata Nygaard (Figs. 150-156)

Observations: Lake Morrison, South-west Tasmania, 2 April 1982.

Comments: Present in high numbers. Previously reported from Europe, Japan, and North and South America (see Nicholls, 1982). For habitat description in Tasmania see King and Tyler (1983).

Mallomonas annulata (Harris and Bradley) Harris (Figs. 157-158).
Harris 1967, J. gen Microbiol. 46, p. 187-188, figs. 6-8, pl. 3, figs. 7-10, pl. 4, fig. 11.

Observations: Gumeracha Weir on the River Torrens in South Australia, 28 May 1982.

Comments: Previously reported from Europe (e.g. Harris, 1967), Japan (Takahashi, 1959), and South America (Durrschmidt, 1980). It has also been observed in Malaysia (Durrschmidt, pers. comm.). Reported here from a mesotrophic environment.

cf. *Mallomonas areolata* Nygaard (Fig. 159).
Nygaard 1949, Kgl. Da. Vid. Selsk. Biol. Skr. 7 (1), p. 117-120, figs. 62, 63.

Observations: Gumeracha Weir on the River Torrens in South Australia, 28 May 1982.

Comments: Only one scale seen. Characters of *M. areolata* and related genera, e.g. *M. corymbosa* and *M. tonsurata*, are variable and it is not possible to identify this scale more closely. Reported here from a mesotrophic environment.

Mallomonas calceolus Bradley (Figs. 160-161).
Bradley 1964. J. gen. Microbiol. 37, p. 322-323, fig. 1, pl. figs. 1-5.

Observations: Sulphide Pool, South-west Tasmania, 4 Nov. 1982.

Comments: Only three scales seen. Previously reported from North and South America, and Europe (see Nicholls, 1982). For habitat description in Tasmania see King and Tyler (1983).

Mallomonas lychenensis Conrad (Fig. 162)

Conrad 1938, Bull. Mus. Hist. Nat. Belg. 14, No. 20.

Observations: Sulphide Pool, South-west Tasmania, 23 Feb. 1983.

Comments: This organism is probably a forma of *M. lychenensis* but may be a closely related, undescribed species. Further TEM work is required to characterize the structure of the scales and determine the true taxonomic position of this organism. *M. lychenensis* has previously been reported only from Europe (e.g. Kristiansen, 1980), but has also been observed in South America (Durrschmidt, pers. comm.). It is characteristic for the species to occur in humic waters. For habitat description in Tasmania see King and Tyler (1983).

Mallomonas mangofera Harris and Bradley (Figs. 163-166).

Harris and Bradley 1960, J. gen. Microbiol. 22, p. 772-773, figs. 41-44, pl. 7, figs. 54, 56, 57.

Observations: Lake Fidler and a small farm dam near Springfield, Tasmania.

Comments: Relatively common in Lake Fidler. Previously reported from Europe, Japan, Bangladesh and North America (see Nicholls, 1982), and has also been observed in South America (Durrschmidt, 1983) and Malaysia (Durrschmidt, pers. comm.). For habitat description in Tasmania see King and Tyler (1982a).

Mallomonas favosa Nicholls (Figs. 167-168).

Nicholls 1984, Can. J. Bot.

Observations: Kulukuluku Billabong, Northern Territory, 8 October 1980.

Comments: Only two scales seen. Previously reported from South America (Durrschmidt, 1983). For habitat description in Australia see Walker and Tyler (1984).

Mallomonas morrisonensis Croome and Tyler (Figs. 169-174).

Croome and Tyler 1983, Brit. phycol. J. 18, p.483-489, figs. 1-10.

Observations: Lake Morrison and Sulphide Pool, South-west Tasmania.

Comments: Described as a result of this study (see Croome and Tyler, 1983c, Appendix III). Of sporadic occurrence. The finger-like projections from the posterior scales are visible under the light microscope. Since the original description scales have been observed with as many as four of these projections (e.g. Fig. 173). The organism has also been observed in Malaysia (Durrschmidt, pers. comm.). For habitat descriptions in Tasmania see Croome and Tyler (1983c) and King and Tyler (1983).

Mallomonas perforata Hickel and Cronberg (Fig. 184).
Hickel and Cronberg (Durrschmidt, pers. comm.).

Observations: Lake Leake, Tasmania, 10 Feb. 1971.

Comments: Only three cells observed. A species which also occurs in Chile and Malaysia (Durrschmidt, pers. comm.). For habitat description in Tasmania see Croome and Tyler (1972).

Mallomonas plumosa Croome and Tyler (Figs. 175-182).
Croome and Tyler 1983, Brit. phycol. J. 18, p. 151-158, figs. 1-18.

Observations: Lake Leake and Tooms Lake in Tasmania, Lake Hume on the border of New South Wales and Victoria, and Yan Yean Reservoir in Victoria.

Comments: Described as a result of this study (see Croome and Tyler, 1983a, Appendix I). Also seen recently in New Zealand by Dr. M. Durrschmidt (pers. comm.). A very distinctive species, perhaps the most distinctive yet described. The cells have a whorl of apical scales with plumes in addition to normal body scales with serrated bristles. The structure of the scales, plumes and bristles possibly provides evidence of a phylogenetic link between the Chrysophytes and the diatoms, as many similarities to the unusual diatom *Corethron* are apparent (Round, pers. comm.). For habitat descriptions in Australia see Croome and Tyler (1972; 1983a) and Croome (1980).

Mallomonas pseudocratis Durrschmidt (in prep.) (Fig. 183).
Durrschmidt (pers. comm.).

Observations: Lake Leake, Tasmania, 10 Feb. 1971.

Comments: Only one cell seen. This organism is yet to be described, but has been found in South America (Durrschmidt, pers. comm.). For habitat description in Tasmania see Croome and Tyler (1972).

Mallomonas splendens (West) Playfair emend. Croome, Durrschmidt and Tyler.
Playfair 1912, Proc. Linn. Soc. NSW, 37, p.512-552, pl. 52, figs. 12,13.
Syn. *Lagerheimia splendens* West 1909

M. splendens f. *splendens*

Observations: Woodford Creek Reservoir in New South Wales, Umbungbung and Kulukuluku Billabongs in the Northern Territory.

Comments: The description of this organism has been emended from observations made during this study, and those made by Durrschmidt from Malaysia (see Croome, Durrschmidt and Tyler, 1984, Appendix VII). There have been light microscope reports of this organisms from New South

Wales (Playfair, 1921), Lakes Hume and Mulwala on the border of New South Wales and Victoria (Viyakornvilas, 1974), Holland (Conrad, 1933), Java (e.g. Huber-Pestalozzi, 1941), India (Philipose, 1953), and Malaya (Prowse, 1962).

M. splendens f. *arnhemensis* Croome, Durrschmidt and Tyler (Figs. 186-189).

Observations: Umbungbung and Kulukuluku Billabongs, Northern Territory of Australia.

Comments: This organism has been described as a result of this study (see Appendix VII) and differs from the type in that the body scales have a conspicuous annular or ellipsoidal depression in the angle of the V-rib.

Mallomonas striata var. *serrata* Harris and Bradley (Fig. 185).
Harris and Bradley 1960, J. gen. Microbiol. 22, p. 761, figs. 13-15, tab. 3, figs. 19, 20, tab. 4, fig. 29.

Observations: Woods Lake, Tasmania, 22 July 1969.

Comments: Only one cell seen. Previously reported from Europe (e.g. Peterfi, 1966), North America (e.g. Wujek *et al.*, 1975), South America (Durrschmidt, 1982), and from Japan and Western Australia (Takahashi, 1978). For habitat description in Tasmania see Buckney and Tyler (1973b).

Mallomonas tonsurata Teiling (Figs. 190-194).
Teiling 1912, Svensk Bot. Tidskr. 4, 277.

Observations: Lake Hume on the border of New South Wales and Victoria, 11 September 1984.

Comments: Present in significant numbers. Previously reported from Europe (e.g. Kristiansen, 1980), North America (e.g. Nicholls, 1982), South America (Durrschmidt, 1980), equatorial and southern Africa (Compere, 1974 and Takahashi, 1978 resp.), and Japan and Korea (Takahashi, 1978). It has also been observed in Malaysia (Durrschmidt, pers. comm.). For habitat description in Australia see Croome (1980).

Mallomonopsis elliptica Matvienko (Figs. 195-199).
Matvienko 1941, Trudy Inst. Bot. Chark. 4, p. 41-47.

Observations: Kulukuluku and Umbungbung Billabongs, Northern Territory, October 1980.

Comments: Full description published in Croome and Tyler (1983b, Appendix II). Previously reported from Europe (e.g. Kristiansen, 1980), North America (Kristiansen, 1975), Japan and Korea (Takahashi, 1978), and equatorial Africa (Compere, 1974). It has also been observed in Malaysia (Durrschmidt, pers. comm.). For habitat descriptions in Australia see Walker and Tyler (1984).

Mallomonopsis tasmanica Croome and Tyler (Figs. 200-204).
Croome and Tyler 1983, Br. phycol. J., 18, p.357-361, Figs.1-6.

Observations: Sulphide Pool, South-west Tasmania.

Comments: Described as a result of this study (see Croome and Tyler, 1983b, Appendix II). Blooms periodically in summer. An unusual species of *Mallomonopsis* in having extremely long bristles (38-140 µm). For habitat descriptions see Croome and Tyler (1983b), King and Tyler (1983).

Paraphysomonas caelifrica Preisig and Hibberd (Figs. 205-209).
Preisig and Hibberd 1982, Nordic J. Bot., p. 412-415, fig. 9, A-L.

Observations: Sulphide Pool in South-west Tasmania, 5 November 1982. See Section 3.4.3.10.

Comments: The cells from Sulphide Pool have shorter spines than those pictured by Preisig and Hibberd, but Preisig has also observed cells with short spines and considers the species to be very polymorphic (Preisig, pers. comm.). Previously reported from England and Denmark (Preisig and Hibberd, 1982b), and has also been observed from Switzerland (Preisig, pers. comm.). For habitat description in Tasmania see King and Tyler (1983).

Paraphysomonas vestita (Stokes) de Saedeleer (Figs. 210-214).
De Saedeleer 1929, Ann. Protistol., 2, p. 177-178.

Observations: Lake Fidler, Lake Morrison, and Sulphide Pool, South-west Tasmania (4 April 1982, 28 May 1976 and 4 November 1982 resp.), small farm dam near Springfield, Tasmania, 7 June 1982, Gumeracha Weir on the River Torrens in South Australia, 28 May 1982.

Comments: An uniquitous species. Previously reported from Europe, North and South America, equatorial and Southern Africa, Bangladesh and Japan (see Preisig and Hibberd, 1982b), and from West Australia (Takahashi, 1978). For habitat descriptions in South-west Tasmania see King and Tyler (1982a: 1983).

Synura australiensis Playfair (Figs. 219-220).
Playfair 1915, Proc. Linn. Soc. NSW., 40, p.315, pl. XLV, figs. 4-5.

Observations: Kulukuluku Billabong in the Northern Territory, Lake Hume on the border of New South Wales and Victoria, and Lake Leake in Tasmania.

Comments: In its cell morphology *S. australiensis* is a distinctive organism, with cells 34-70 µm by 6.0-7.5 µm. However, in its scale structure it is very similar to *S. petersenii* (see below)

except that the scales are much more elongate. *S. australiensis* could be designated as a variety of *S. petersenii*. This step is not taken here, however, because there is some doubt about the status of *S. petersenii* itself: it may be synonymous with *S. granulosa* (see *S. petersenii* below and Croome and Tyler, 1984e, Appendix X). A thoroughgoing review of the genus is required to resolve these issues.

S. australiensis is reported here for the first time since its original description (Playfair, 1915). However, similar scales were pictured (as *S. petersenii*) by Wujek and Van Der Veer (1976) from the Netherlands.

Synura curtispina (Petersen and Hansen) Asmund (Figs. 215-216).
Asmund 1968, Hydrobiologia 31, p. 506-508.

Syn. *Synura spinosa* Korsh. f. *curtispina* Petersen and Hansen 1956.

Observations: Lake Fidler and Lake Leake, Tasmania, Kulukuluku Billabong, Northern Territory, and Lake Hume on the border of New South Wales and Victoria.

Comments: The scales observed were smaller than those usually described for this species, but Durrschmidt (1980) reported scales of similar size from Chile. Durrschmidt has also observed this species in Malaysia (pers. comm.). For habitat descriptions in Australia see King and Tyler (1982a), Walker and Tyler (1984), Croome and Tyler (1972), and Croome (1980).

Synura echinulata Korshikov (Figs. 217-218).
Korshikov 1929, Arch. Protist., 67, p. 253-290.

Observations: Kulukuluku Billabong, Northern Territory, 8 October 1980.

Comments: Only one cell seen. Previously reported from Europe, North America, equatorial Africa and Japan (see Takahashi, 1978). It has also been observed in Malaysia (Durrschmidt, pers. comm.). For habitat description in Australia see Walker and Tyler (1984).

Synura petersenii Korshikov (Figs. 221-225).
Korshikov 1929, Arch. Protist., 67, p.283, pl. 11, figs. 54-58.

Observations: Lake Morrison, Lake Fidler, Risdon Brook Dam, Arthurs Lake, Woods Lake, Lake Leake and the Macquarie River in Tasmania, Kulukuluku and Umbungbung Billabongs in the Northern Territory, Lake Hume on the border of New South Wales and Victoria, and Gumeracha Weir on the Torrens River in South Australia.

Comments: An ubiquitous species. From light microscope

observations it is likely that *S. petersenii* Korshikov 1929 is synonymous with *S. granulosa* Playfair 1915, a species which Playfair described from near Lismore in New South Wales. Playfair stated that *S. granulosa* was the most common *Synura* of this country: this investigation has shown that *S. petersenii* is the most common species in Australia. Playfair's type material of *S. granulosa*, which is held by the National Herbarium of New South Wales, has been examined by electron microscopy. However, no well preserved chrysophyte scales have been found to date, and the question of the relationship between *S. petersenii* and *S. granulosa* remains to be answered.

S. petersenii has previously been recorded from Europe, North and South America, Japan and Korea (see Durrschmidt, 1980), and equatorial Africa (Compere, 1974). It has also been observed in Malaysia (Durrschmidt, pers. comm.). For habitat descriptions in Australia see Walker and Tyler (1984), King and Tyler (1982a: 1983), Buckney and Tyler (1973), Croome and Tyler (1972) and Croome (1980).

Synura spinosa Korshikov (Figs. 226-227).
Korshikov 1929, Arch. Protist., 67, p. 281, pl. 11, figs. 38-41.

Observations: Lake Fidler, South-west Tasmania, and Lake Hume on the border of New South Wales and Victoria.

Comments: Previously recorded from North and South America, Europe, equatorial Africa and Japan (see Durrschmidt, 1980). It has also been observed in Malaysia (Durrschmidt, pers. comm.). For habitat descriptions in Australia see King and Tyler (1982a) and Croome (1980).

3.6.4 Comments

The global distribution of Chrysophytes, and the Mallomonadaceae in particular, has been discussed recently by Kristiansen (1981). Most studies have been made in the northern hemisphere - mainly North America, western Europe, and Japan - and only a handful in the southern hemisphere. (Only one previous electron microscope report on Chrysophytes has been made from Australia: Takahashi (1978) observed *Mallomonas papillosa, M. striata, Mallomonas* spp., and *Paraphysomonas vestita* in Western Australia). Most studies have been made in regions with cool-temperate or cold climates, and few collections have been made in tropical areas. However, the collections which have been made in the tropics indicate a very rich

flora in these regions, and the old belief that the Chrysophyceae are specially attached to cold areas must be abandoned. The old idea that Chrysophytes are characteristic of oligotrophic and mesotrophic waters also has to be discarded, following the recent discovery of thirty three species of Mallomonadaceae in Denmark's eutrophic Lake Tystrup So (Kristiansen, 1984).

Kristiansen (1981) considers that more than fifty per cent of the Mallomonadaceae presently described are cosmopolitan, being found in Europe, North America and Japan, and from both arctic and tropical regions. A further twenty per cent are known exclusively from Europe. Another group comprises individuals of more scattered occurrence, with few records in various parts of the world.

Of the twenty seven members of the Mallomonadaceae found in Australian waters during this survey (see Section 3.6), three are new species (*Mallomonas plumosa, M. morrisonensis* and *Mallomonopsis tasmanica*), and a further seven are reported from the southern hemisphere for the first time. Most of the species observed can be considered cosmopolitan, but some are of extremely limited distribution, having been reported previously from a few localities in Europe, or reported now from one or two localities in Australia.

More than half the species observed were present in the lakes adjacent to the Gordon River in South-West Tasmania: sixteen species occurred in these lakes, and nine of these were seen nowhere else. Three species (*Mallomonas favosa, Mallomonopsis elliptica* and *Synura echinulata*) were seen only from the tropical Northern Territory. Four species (*Mallomonas splendens, Synura curtispina, S. australiensis* and *S. petersenii*) were found throughout the continent from tropical to cool-temperate areas. The remainder were limited in distribution and occurrence in the southern part of the continent. Several observations were of one or two cells, or even one or two scales, only and the number of species found, and their distribution, will no doubt increase as more samples are viewed. Nevertheless, many dozens of samples were viewed during this study, and the list in Section 3.6.3 is considered to be a good indication of the presence and abundance of the organisms in Australia.

One species, *Synura petersenii*, is of economic importance in Australia in that it blooms during the colder months in several of the reservoirs which store and supply industrial and domestic water to Adelaide in South Australia. The blooms produce severe taste and odour problems, and it is not unusual for many thousands of dollars to be

spent on the dosing of these reservoirs with copper sulphate for the control of this organism.

Lake Fidler and Sulphide Pool

Fourteen species of Mallomonadaceae were observed in Lake Fidler or Sulphide Pool. This represents fifty per cent of the species found throughout Australia during this survey, and there is no doubt that the lakes are two of Australia's foremost localities for these organisms. Such humic waters have been found elsewhere to be similarly endowed with Chrysophytes: many species were seen in thirty five Finnish brown-water lakes by Ilmavirta (1980) for instance, who concluded in another paper (Ilmavirta, 1983) that flagellated Chrysophytes and Cryptomonads were dominant in more darkly coloured waters. In Lake Fidler flagellates comprised some fifty per cent of the species observed during this study, and were certainly dominant in terms of biomass. Flagellates were also dominant in terms of biomass in Sulphide Pool, but comprised only twenty five per cent of the species observed due to the greater number of desmids present in this more sheltered, pond-like water body.

4. DISCUSSION

4.1 INTRODUCTION

The discussion below concerns several aspects of the study documented herein. In particular, it compares Lake Fidler and Sulphide Pool with similar lakes throughout the world, especially concerning the organisms of the microbial stratifications, their biomass and productivity, and their relationship to the underwater light climate and supply of nutrients. A section on the permanence of the meromixis in Lake Fidler and Sulphide Pool has been included, as has a section on the preliminary observations of Australian Chrysophytes documented herein. It is a relatively concise discussion of the more interesting facets of the investigation: considerably more details and comments on the data collected during this study are given in Section 3.

4.2 MICROBIAL STRATIFICATIONS IN OTHER LAKES

There is no doubt that Lake Fidler and Sulphide Pool are superb examples of the meromictic condition. With their peculiar origins, their dystrophy, and their sharp partitioning of upper and lower waters, they are two very interesting meromictic lakes. In addition, they contain many organisms not recorded previously in the southern hemisphere, and several new to science. (Two of the latter have been described during the course of this study - Croome and Tyler, 1983b,c). However, there is also no doubt that it is the intense microstratifications of organisms at depth which make these two lakes particularly interesting, and unique.

Numerous stratified lakes throughout the world contain discrete layers of microorganisms at the upper levels of their bottom waters. Frequently the dominant organisms at such depths are photosynthetic bacteria, but often other bacteria form an important part in the layering, as do cyanobacteria and, occasionally, eukaryotic algae. The contribution made by these organisms to the primary productivity of a particular water body is usually low, as little light reaches the depths at which they occur, but in some lakes they play a major role in the fixation of inorganic carbon.

Given the number of lakes in the world exhibiting stratification of organisms at depth, in comparison with the total number of lakes *per se*, there is a surprising amount of data available for discussion. As the amount of data is so large (and complex), discussion here will be limited

to those lakes most similar to Lake Fidler and Sulphide Pool, and those displaying microbial stratifications of particular interest. The former group comprises meromictic lakes in which members of the Chlorobiaceae are dominant at the mixolimnetic/monimolimnetic boundary. These lakes are Lake Faro in Sicily (Truper and Genovese, 1968; Sorokin and Donato, 1975), Fayetteville Green Lake in New York State (Culver and Brunskill, 1969), Repnoe Lake in the USSR (Chebotarev *et al.*, 1973; Gorlenko *et al.*, 1973), Lake Gel-Gel in the USSR (Dubinina *et al.*, 1973), Lake Veisovo and Pomyaretskoe Lake in the USSR (Gorlenko *et al.*, 1974a:b), Waldsea Lake in Saskatchewan (Hammer *et al.*, 1978; Lawrence *et al.*, 1978), Lake Vila in Spain (Guerrero *et al.*, 1980), Lake Sakovo in the USSR (Gorlenko and Chebotarev, 1981), Lake Mary in Wisconsin (Parkin and Brock, 1980a) and Knaack Lake in Wisconsin (Parkin and Brock, 1980b, 1981). Of these, Lake Mary and Knaack Lake have another similarity to Lake Fidler and Sulphide Pool in that they are dystrophic. The second group, made up of those lakes which display other microbial stratifications of particular interest, comprises Lake Haruna in Japan (Takahashi and Ichimura, 1968), Lake Konon"er in the USSR (Gorlenko and Kuznetsov, 1971), Lakes Burke, Wintergreen, Duck and Cassidy in Michigan (Caldwell and Tiedje, 1975), Lake Lesnaya Lamba in Karelia (Dubinina and Kuznetsov, 1976), Solar Lake in Sinai (Cohen *et al.*, 1977), Lake Tollari, Myakhkli and Viytina in Estonia (Gorlenko and Lokk, 1979), Lakes Peter, Paul, Rose, Mirror and Fish in Wisconsin (Parkin and Brock, 1980a), Lake Vechten in the Netherlands (Blaauboer, 1982; Steenbergen, 1982; Steenbergen and Korthalis, 1982), and Lake Holmajon in Finland (Lindholm *et al.*, 1984).

The microbial stratifications typically occur across the oxic/anoxic interface present at depth in these lakes. In meromictic lakes the depth of this interface corresponds to that of the chemocline; in lakes which are thermally stratified it corresponds to that of, or just below, the thermocline. (An important variation on this is seen in Knaack Lake, which will often be used herein for comparison with Lake Fidler and Sulphide Pool. Knaack Lake is meromictic but the microbial stratification within it forms not at the chemocline, but at a thermocline which forms annually in the upper waters of the lake). At these depths there is usually a distinct change from oxygenated conditions above to anaerobic sulphuretted conditions below. The oxic/anoxic boundary is usually stationary, but has been reported to move in some lakes, e.g. in Solar Lake the boundary undergoes a diurnal migration, and in Knaack Lake it migrates slowly downwards throughout the stratification period.

The depth of the microbial zone varies from lake to lake. In the meromictic lakes containing Chlorobiaceae, for example, the depth ranges from 1.5-3.5 metres in Knaack Lake, to 7 metres in Lake Waldsea, to 18-20 metres in Fayetteville Green Lake, to 20-30 metres in Lake Gel-Gel. Despite these large differences in depth, the organisms receive similar amounts of light, the microbial zone in most of the lakes receiving less than one per cent of the light present at the lake surface. The microbes in Knaack Lake, for instance, receive 0.03-0.10 per cent of surface light (light penetration is restricted by dissolved humic material), those in Lake Waldsea 1 per cent, and those in Fayetteville Green Lake something less than 0.1 per cent.

Historically, it has been the photosynthetic bacteria which have generated most interest in these lakes, and in early works in particular, information on other organisms is scant. However, it is sometimes possible to deduce the quantity and distribution of other bacteria and algae in these early works, and later works have tended to place less emphasis on the photosynthetic bacteria *per se* and more on the whole spectrum of organisms present. Of the meromictic lakes listed above which contain Chlorobiaceae, no other significant bacterial or algal component has been reported in the microbial stratification of Faro, Reproe, Gel-Gel, Pomyaretskoe, Veisovo, Waldsea, or Vila. In Fayetteville Green Lake an algal population peak was found just above the redoxcline on one occasion only. In Lake Mary Chlorophyll *a* concentrations increased with depth and maximum algal production was found only one metre above the photosynthetic bacterial layer, but the authors made no mention of algal stratifications. A similar situation was reported for Lake Sakovo. In Knaack Lake algal concentrations increased with depth and were at a maximum just above the photosynthetic bacterial zone. The predominant algae were *Oocystis*, *Gloeocystis*, *Haematococcus*, *Staurastrum* and *Scenedesmus*, and many unidentified green flagellates and desmids were also observed. Cyanobacteria, mainly *Anabaena*, were also present. However, the authors again made no comment concerning the stratification of these organisms.

Many of the papers on the thermally stratified lakes contain similar reports. In their paper on the 10 Japanese lakes, Takahashi and Ichimura (1968) gave details of significant algal populations within 1-3 metres of the layer of photosynthetic bacteria. However, they made no mention of any stratification of the algae, and the impression is gained that they were not a permanent part of the microbial array. The exception to this was a sampling in Lake Haruna when "an enormous population of photosynthetic

flagellates (*Cryptomonas* spp.) appeared at the bottom of the thermocline, near the base of the euphotic zone, and in contact with the H_2S zone." In their paper on 5 lakes in Wisconsin, Parkin and Brock (1980a) showed chlorophyll profiles for their thermally stratified lakes which indicated a Chlorophyll *a* maximum near the top of the photosynthetic bacterial population, but again no comment was made about the participation of the algae in the microbial arrays of these lakes.

In other papers, significant details of microstratifications within the microbial array of thermally stratified lakes are given, and these are worth considering in detail. In Solar Lake, Cohen *et al.* (1977) found several different layers of phototrophic organisms during winter stratification. (Solar Lake is an unusual monomictic lake in having a period of holomixis in summer). In the metalimnion a dense population of purple sulphur bacteria developed, the upper hypolimnion was dominated by a population of a green sulphur bacterium, and beneath this was a dense layer of cyanobacteria. The vertical distribution of the two types of photosynthetic bacteria in Solar Lake fitted perfectly the description by Van Niel (1963) "The green bacteria have a greater tolerance for H_2S than do the purple sulfur bacteria. The former are therefore apt to occur closer to the source of H_2S, which is usually generated by the biological activity (sulphate reduction) in the bottom sediments. Hence mass developments are frequently stratified with the purple above the green sulfur bacteria." However, as Cohen *et al.*, observed, examples of two different plates of photosynthetic bacteria are rare (Gorlenko and Libeva, 1971; Dubinina and Kuznetsov, 1976), and in most stagnant lakes only a single plate is observed. They conclude that the occurrence of such a double plate in Solar Lake is a consequence of the extremely high irradiation (*sic*) in the lake, which allows efficient light intensities to reach the H_2S-rich layer at 4.5-5.0 metres. In fact light penetrates right to the bottom of the lake (6 metres).

In Lesnaya Lamba, Dubinina and Kuznetsov (1976) found a distribution of organisms dependant on the concentration of iron, manganese, oxygen and light. They found three strata of different iron bacteria in the oxygenated waters immediately above the chemocline, and phototrophic bacteria in the anaerobic waters. In all, the distribution of 9 phototrophic bacteria was characterized; purple sulphur bacteria were dominant close to the chemocline, and green sulphur bacteria were dominant at greater depth. As with Solar Lake, few algae were present, they were confined to the surface waters, and they played no part in the microbial stratification at depth.

In Konon"er Lake, Gorlenko and Kuznetsov (1971) found a distribution of individual species controlled by H_2S content, pH and penetration of light. A layer of iron- and manganese-oxidizing bacteria were present above a layer of cyanobacteria in oxygenated waters, and below the oxic/anoxic interface were layers of several photosynthetic bacteria. Again, algae were present only near the surface of the lake and did not participate in the microbial stratification.

In Lake Vechten, Steenbergen and Korthals (1982) found photosynthetic bacteria in the sulphide-carrying waters, cyanobacteria above these in the microaerophilic strata, and above these in the uppermost zone of the metalimnion (also under microaerophilic conditions) the eukaryotic algae *Mallomonas caudata* at up to 500-1,000 cells/mL (see Blauuboer, 1982), and *Cryptomonas* and *Rhodomonas* at up to 100 cells/mL. Algae were also present in the surface waters of the lake, but those at depth were a separate population and formed a definite association with the microbial stratification.

In Lake Holmsjon, Lindholm *et al.* (1984) found the development of a temporary meromixis, with a microbial stratification at depth during summer. The stratification consisted of *Chlamydomonas* very close to and even within the anoxic zone, purple photosynthetic bacteria near the top of the anoxic zone, and green photosynthetic bacteria below these. Cell concentrations of *Chlamydomonas* were sufficiently high to colour the water light green, and cause a peak in the chlorophyll and primary production profiles in the lake.

Hence, although there have been many studies of microbial stratifications at the oxic/anoxic interface in lakes, the participation of eukaryotic algae in such stratifications has only been demonstrated clearly on three occasions, in Lakes Haruna, Vechten and Holmsjon. Reports of several other lakes, e.g. those in Wisconsin by Parkin and Brock, suggest that some of the algae present may participate in the microbial stratifications within them also, but firm evidence of this is lacking.

4.3 LOCATION, STRUCTURE AND PHYSIOLOGY OF THE ORGANISMS IN THE MICROBIAL STRATIFICATIONS OF LAKE FIDLER AND SULPHIDE POOL

Given the above, the microbial distribution at depth in Lake Fidler is unique. Six organisms occur persistently about the redoxcline: above it, the minute green alga *Scourfieldia caeca* occurs at concentrations up to 860,000 cells/mL, the larger euglenoid *Trachelomonas volvocina* at up to 2,750 cells/mL, and the colourless sulphur bacteria *Beggiatoa* and cf. *Achromatium* at up to 38,000 filaments and 140,000 cells/mL respectively; below it, the green photosynthetic bacterium *Chlorobium* is present at up to 40×10^6 cells/mL; and either above or below it, the ectosymbiotic consortium *"Chlorochromatium aggregatum"* peaks at concentrations up to 640,000 consortia/mL. Several other algae and bacteria take part in the stratification on an occasional basis.

The distributions of these organisms overlap to a considerable extent. Nevertheless, the population maximum of any one organism is usually discrete.

The identity of the minute flagellate alga *Scourfieldia caeca* was one of the enigmas of Lake Fidler at the beginning of this study. Colloquially known as "Matchbox" from its appearance while swimming, its identification was of particular interest to those "algal-based" limnologists who had participated in work on the lake. *S. caeca* has previously been reported only from temporary pools and ponds in Europe. Its discovery as a permanent and significant component of the microbial stratification about the redoxcline in a meromictic lake in Tasmania is surprising. However, it gross morphology, distinctive mode of locomotion, and cellular ultrastructure leave no doubt that it is a member of this taxon.

S. caeca maintains a highly stratified, diurnally stable distribution above the redoxcline, occurring at up to 860,000 cells/mL, with maximum cell numbers often present in a layer less than 0.05 metres thick. It is a constant member of the microaerophilic zone, peaking 0.05-0.30 metres above the redoxcline, at light intensities less than 0.5 per cent of that incident at the lake surface.

As mentioned above, the ultrastructure of *S. caeca* was found to be similar to that reported previously (by Manton, 1975). However, the cells examined by Manton were taken from culture, and two significant differences were noted between her cultured cells and those examined from Lake Fidler. Firstly, the chloroplasts of the Lake Fidler cells were

relatively much larger, and contained many more thylakoids, presumably in response to the low light levels at depth in the lake. Secondly, ultrastructural observations of the crescentic "keel" present in the chloroplast of the Lake Fidler cells agreed more closely with those of earlier workers using light microscopes to observe wild cells, than with the electron microscope observations of Manton using culture material. Whereas previous workers had described the keel to constitute a single large starch grain, Manton said that starch was present only at its centre and the remainder was of an amorphous material. Observations made of cells from Lake Fidler showed the keel to be more homogenous.

The euglenoid alga *Trachelomonas volvocina* is also a constant member of the microbial stratification, with population maxima of 40-2,750 cells/mL at 0.05-0.75 metres above the redoxcline. *T. volvocina* thus occurs in the microaerophilic zone, at light intensities regularly less than 1 per cent of surface values.

The taxonomy of *Trachelomonas* is currently based on the morphology of the cell envelope and the organism here has been identified on this basis. Ultrastructurally, it is also similar to *T. volvocina*, except that the cell contains numerous small discoid chloroplasts without pyrenoids instead of two large chloroplasts with inner pyrenoids, as reported for *T. volvocina* by Leedale (1975).

The flagellate algae of the microbial stratification of Lake Fidler are presumably present at depth in response to the supply of nutrients. Nutrient analyses were not carried out during this study, but some were reported by King and Tyler (1982a, 1983). They found that nitrate analyses could not be completed due to procedural difficulties with sulphur precipitates, but did analyse for phosphorus on several occasions. They reported up to 350 µg/L of orthophosphate in the bottom waters of Lake Fidler and 145 µg/L in Sulphide Pool, but found less than 10 µg/L of orthophosphate near the surface of the lakes. As might be expected, the discontinuity between the two extremes occurred across the redoxcline. Detail is lacking, but from the nature of the discontinuity as shown in the data of King and Tyler, it appears that significant amounts of phosphorus, at least, could be available to the flagellate algae in the microaerophilic zones of the two lakes.

Population maxima of two organisms taken to be colourless sulphur bacteria are found in the microaerophilic zone of Lake Fidler. Given our present state of knowledge of the colourless sulphur bacteria, the group should be described, rather than defined, as containing those organisms which can or are believed to be able to derive metabolically useful energy from the oxidation of reduced inorganic sulphur compounds (Kuenen, 1975). The organisms are commonly present under aerobic conditions at oxic/anoxic interfaces, where they participate in the oxidation of sulphide to sulphate. In Lake Fidler the colourless sulphur bacteria *Beggiatoa* and cf. *Achromatium* are present as consistent members of the microbial array. During this study a population of *Beggiatoa* peaked in the 0.40 metres above the redoxcline at concentrations from 700-38,000 filaments/mL. Similarly, a population of cf. *Achromatium* peaked at equivalent depth at 16,000-140,000 cells/mL.

Achromatium is one of the least-known colourless sulphur bacteria, and virtually nothing is known of its physiology or the role of sulphide in its metabolism; most species of *Beggiatoa* known thus far are heterotrophic organisms whose growth can be stimulated by the presence of sulphide (Kuenen, 1975). Their presence in the microaerophilic zone of Lake Fidler is in accord with their known distribution at oxic/anoxic interfaces, except that *Achromatium* usually contains calcium carbonate granules, and thus is relatively dense and confined to solid substrates.

The location of large populations of *Beggiatoa* and cf. *Achromatium* immediately above the redoxcline begs the question as to whether dissolved sulphide may be present, enabling the organisms to grow chemolithotrophically. (Before most strains were shown to be heterotrophic, *Beggiatoa* was regarded as the archetypal chemolithotrophe). Co-existence of H_2S and O_2 at depth have been demonstrated, particularly in lakes in which the oxic/anoxic boundary layer migrates, e.g. Solar Lake (Cohen *et al.*, 1977). However, most of the studies of the O_2/H_2S interface in stratified waters have not had a sufficiently fine depth resolution to determine the co-existing concentrations of the two compounds accurately, and most studies have not, in any case, applied sufficiently precise analytical methods (Jorgensen, 1982). Chemical analyses during this study showed no dissolved sulphide present above the redoxcline in Lake Fidler. If sulphide is present in significant amounts in the microaerophilic zone, then it is being oxidized so rapidly by either chemical or biological transformations that it is not showing up in the chemical analyses. It is more likely that significant amounts of sulphide are not present, and the colourless

sulphur bacteria are living heterotrophically. Nonetheless, the question of the metabolism of these two organisms, and the culturing and identification of cf. *Achromatium*, are certainly meaningful projects for the future.

The water immediately below the redoxcline in Lake Fidler is coloured bright green by a large population of the green photosynthetic bacterium *Chlorobium*. This layer of green water is perhaps the single most impressive feature of the lake. The *Chlorobium* population comprises 3 forms: *C. limicola*-like rods, *C. vibrioforme*-like curved cells, and a twisted form of many cells joined end to end. During the study cell numbers of 40×10^6 cells/mL were recorded. Maximum concentrations were usually found at the depth at which dissolved sulphides were first detected, within 0.5-0.10 metres of the redoxcline. The population maximum was often very sharp, but on occasion the cells were more evenly distributed in the water column. Surprisingly, significant numbers of this obligate anaerobe were found in the microaerophilic zone above the redoxcline, at oxygen concentrations up to 2.0 mg/L. The cells are presumed to be "stragglers" lost from their optimum position in the water column due to inefficient depth regulation. Such distributions have not been reported previously, but this is taken to be a reflection of the close sampling interval used during this study.

The meromictic lakes listed above have all been found to contain members of the Chlorobiaceae, usually *Chlorobium* or *Pelodictyon*, as the dominant organisms of their photosynthetic bacterial populations. Most of them, however, contain brown forms of the Chlorobiaceae, e.g. *Chlorobium phaeobacteroides* and *Pelodictyon phaeum*. A green *Chlorobium*, *C. chlorovibrioides*, has been found as the dominant organism in one lake only: it was recorded in Lake Pomyaretskoe at concentrations up to 46×10^6 cells/mL (Gorlenko *et al.*, 1974b). Lake Fidler is therefore unusual in having green forms of Chlorobiaceae dominant in its photosynthetic bacterial population. (See below for discussion of the type of photosynthetic bacteria present in lakes in relation to the underwater light climate).

The ectosymbiotic consortium "*Chlorochromatium aggregatum*", in which cells of *Chlorobium* are arranged in an envelope around a large, colourless, motile bacterium of unknown physiology and taxonomic position, is one of the more intriguing "organisms" present in Lake Fidler.

The consortium has been found in large numbers in several other stratified lakes. In Lake Sakavo (Gorlenko and Chebotarev, 1981) and Lesnaya Lamba (Dubinina and Kuznetsov, 1976) it occurred at the same depth as free-living green photosynthetic bacteria, in numbers up to 420,000 and 100,000 consortia/mL respectively. In Lake Nesoytjern it was recorded at 30,000 consortia/mL (Blakar, 1979), and in Burke Lake it comprised 10 per cent of the photosynthetic bacterial community (Caldwell and Tiedje, 1975). It has also been reported in Lakes Mary and Rose (Parkin and Brock, 1980a) and in Estonian Lakes (Gorlenko and Lokk, 1979). In all cases, it has been reported as present under strictly anaerobic conditions.

In Lake Fidler concentrations of "*C. aggregatum*" up to 640,000 consortia/mL have been recorded, the largest population maximum reported yet for this "organism." The population usually peaks in a layer 0.10 metres thick, but the location of this layer varies considerably with respect to the redoxcline. During this study it was found up to 0.25 metres above the cline at a redox potential of 310 mV and a dissolved oxygen concentration of 0.4 mg/L, and as far as 0.40 metres below it at a redox potential of -100 mV and dissolved sulphide concentration of 30 mg/L.

In the other lakes for which this consortium has been reported (see above), it has been found under strictly anaerobic conditions. In Lake Fidler it has been found on several occasions under either aerobic or anaerobic conditions. Such a distribution is interesting in the light of discussion as to the structure and function of the two members of the consortium. This subject has been discussed recently by Pfennig (1980). Firstly, only green sulphur bacteria are involved in symbiotic associations such as "*C. aggregatum*". The green sulphur bacteria carry their photosynthetic electron transport system exclusively in the cytoplasmic membrane associated with the chlorobium vesicles, and the first oxidation product of sulphide, elemental sulphur, is immediately released extracellularly. It is postulated that the sulphur is then directly available to the central symbiont as an electron acceptor. In contrast, the purple sulphur bacteria store the elemental sulphur in globules inside the cells, and are therefore not suited to such ectosymbiotic associations. The metabolic interdependence of the two symbionts is further demonstrated by their synchronous growth and division, and also by the fact that the *Chlorobium* symbionts effect a positive phototactic response in the motile nonphototrophic central bacterium: when grown in culture, the consortia

gradually accumulate at the illuminated side of the culture vessel. It is most likely, then, that the central organism is a sulphate or sulphur-reducing bacterium which utilizes the elemental sulphur produced extracellularly by the photosynthetic bacteria. The photosynthetic bacteria, in turn, use the dissolved sulphide produced by the activities of the central bacterium as an electron donor for their anoxygenic photosynthesis, and also gain a motility which enables positive phototactic responses.

The discovery of large population maxima of *"C. aggregatum"* in both the oxic and anoxic zones of Lake Fidler in no way detracts from the above hypotheses. In fact, it supports them. When it is present in the anoxic zone the metabolic activity of the consortium could be just as supposed above. Its presence in the microaerophilic zone is most likely due to a positive phototactic response, and although the reducing conditions required for anaerobic photosynthesis would not be present outside the consortium, they could well prevail between the cells within it. The consortium is highly motile, and the only "organism" to exhibit a markedly different distribution from month to month. Unfortunately, *"C. aggregatum"* was not present in significant numbers on the occasion of the night sampling of Lake Fidler reported herein. However, it was present on a night sampling on 13 April 1983, when maximum concentrations were observed at 3.55 metres, the depth about which maximum values were recorded for a daylight sampling the previous day (Fig. 41). Marked diurnal variations in depth distribution are therefore unlikely.

Why *"C. aggregatum"* should be found high in the microaerophilic zone in one month, and well into the sulphide zone in another, is not known. Its location is presumably a response to light, redox potential, and sulphur distribution, but there is no clear indication of why it is located at a particular depth on a particular sampling occasion. Certainly, this is one of several intriguing areas for further study.

The structure of *"C. aggregatum"* from Lake Fidler is similar to that found by previous workers, with cells of *Chlorobium* enveloping and being in close contact with a large central bacterium. It is also similar in its ultrastructure, the photosynthetic symbionts possessing distinct chlorobium vesicles, and the central bacterium being covered in a layer of cups which presumably help to keep the consortium together (Figs. 101 and 104). In one ultrathin section (Fig. 107) the central bacterium contained two vesicles near the cell membrane. Such vesicles have not been reported previously, and their function is unknown.

Given the compactness of the microbial array (on occasion as little as 0.30 metres) it is not surprising that the profiles of the individual organisms overlap to a considerable extent and, on occasion, population maxima may occur at the same depth, e.g. in the very first sampling on 31 January 1981 (Fig. 31) the maxima for *T. volvocina*, *S. caeca*, cf. *Achromatium* and "*C. aggregatum*" were all around 0.20 metres above the redoxcline. There is no set ranking of the organisms through the stratification, except that the algae and colourless sulphur bacteria predominate above the redoxcline, and the photosynthetic bacteria below it. There is a tendency for the algae to be present nearer the top of the array, but on several occasions *S. caeca* peaked at the same depth as, or below, one or both of the colourless sulphur bacteria. With the exception of *Chlorobium* and *Beggiatoa*, the organisms of the stratification are motile, and their distribution at any one sampling no doubt indicates the location of their optimum growth conditions on that occasion.

Two other algae were also part of the microbial array at times during this study. The Cryptophycean *Cryptomonas* was observed on five sampling occasions at up to 350 cells/mL. As it is a far more significant organism in Sulphide Pool, its physiology and participation in the stratifications of the two lakes will be discussed below. The Chrysophycean *Synura petersenii* was also observed in the array, but on one occasion only. Other algae have been observed in the array, but are taken to be organisms sedimenting from the upper waters of the lake, e.g. *Ankistrodesmus falcatus* var. *mirabilis*, *Rhizosolenia eriensis* and *Fragilaria ulna*. Other bacteria have also been observed. In particular a large bacterium was found occasionally at the same depth as the population maximum of *Chlorobium*. The bacterium is possibly photosynthetic but has not been identified at the time of writing.

In summary, six organisms are constantly involved in the microbial stratification about the redoxcline in Lake Fidler. The algae *Trachelomonas volvocina* and *Scourfieldia caeca* are present in the microaerophilic zone, as are the colourless sulphur bacteria *Beggiatoa* and cf. *Achromatium*; the green photosynthetic bacterium *Chlorobium* is present at maximum concentrations immediately beneath the redoxcline; and the ectosymbiotic consortium "*Chlorochromatium aggregatum*" is present either above or below the redoxcline. In addition, the algae *Cryptomonas* and *Synura petersenii* participate in the stratification from time to time, as do several other bacteria. Other algae are found occasionally at the depth of the microbial array, but are taken to be sedimenting from the surface waters of the lake.

The composition of the microbial array in the more sheltered, pond-like Sulphide Pool is less complex than that of Lake Fidler in that it comprises algae and photosynthetic bacteria only, no colourless sulphur bacteria being observed during this study. Again, the dominant organism is *Chlorobium*. Its population is less sharply defined than that in Lake Fidler but again is usually at a maximum immediately below the redoxcline. The consortium *"C. aggregatum"* also occurs in Sulphide Pool (as from April 1982), having possibly been introduced on sampling equipment previously used in Lake Fidler. The algae present play a much greater role than those in Lake Fidler: *S. caeca* and *Cryptomonas* are the dominant species, but *T. volvocina, Euglena,* and others occur occasionally, as do several unidentified bacteria.

Cryptomonas has been observed as a constant component of another microbial stratification, that of Lake Vechten (Steenbergen and Korthals, 1982), where it exists under microaerophilic conditions at up to 100 cells/mL. (By contrast, populations of *Cryptomonas* in Sulphide Pool have been recorded as high as 48,000 cells/mL). Other workers (Haffner *et al.*, 1980) have found *Cryptomonas* to have a very low chlorophyll/volume ratio, and observed it congregating around colloidal masses of organic matter. They concluded that in areas where the relationship between the depth of the euphotic zone and that of the mixing depth varies with time *Cryptomonas* does not rely solely on photosynthetic carbon uptake, but also utilizes heterotrophic processes. Such could well be the case for *Cryptomonas* in the highly dystrophic Sulphide Pool, and for all the algae participating in the stratifications of the two lakes, as all persistently receive less than 1% of the light at the surface of the lakes, the value below which net photosynthesis is conventionally assumed to be insignificant (but see below).

4.4 BIOMASS OF THE MICROBIAL STRATIFICATIONS

One of the most striking features of the microbial arrays of Lake Fidler and Sulphide Pool is the large numbers of organisms present. The concentrations of up to 40×10^6 cells/mL for *Chlorobium*, for instance, are amongst the highest ever recorded for photosynthetic bacteria. In comparison, Gorlenko *et al.* (1974b) have reported 46×10^6 cells/mL of *Chlorobium chlorovibrioides* in Pomyaretskoe Lake, and Sorokin and Donato (1975) and Gorlenko *et al.* (1973) have reported 40×10^6 and 33×10^6 cells/mL of *Chlorobium plaeobacteroides* in Lake Faro and Repnoe Lake respectively.

Only one group of workers has recorded bacteriochlorophyll levels as high as those seen in Sulphide Pool (up to 4,000 µg/L; King & Tyler, 1983): Lindholm *et al.* (1984) reported that Bacteriochlorophyll *d* concentrations in Lake Holmsjon "reached values as high as 4,000 µg/L." Prior to this report, the highest value recorded was by Lawrence *et al.* (1978) who found Bacteriochlorophyll *d* levels in Waldsea Lake up to 2,325 µg/L. Takahashi and Ichimura (1970) recorded up to 1,000 µg/L of bacteriochlorophyll in a mixed population, and Parkin and Brock (1981) recorded up to 550 µg/L of Bacteriochlorophyll *d* in Knaack Lake.

On an areal basis the Bacteriochlorophyll *d* values for Lake Fidler ranged from 320-700 mg/m^2 and for Sulphide Pool from 200-600 mg/m^2. The highest values previously reported are 700 mg/m^2 from Waldsea Lake and 590 mg/m^2 from Lake Harutori in Japan (Takahashi and Ichimura, 1970). (The latter authors gave a theoretical maximum for total chlorophyll of 800 mg/m^2). No estimates were made for Lake Holmsjon by Lindholm *et al.* (1984). Given the extremely high cell counts of the relatively large photosynthetic bacteria in Lake Fidler and Sulphide Pool, larger bacteriochlorophyll values on an areal basis might have been expected. However, compared to many lakes, the population strata of *Chlorobium* in Lake Fidler and Sulphide Pool are narrow, and biomass is therefore smaller on an areal basis.

Scourfieldia caeca is present in the lakes in concentrations up to 1.3×10^6 cells/mL. No chlorophyll determinations were made on samples containing this many algae, but an analysis of a sample containing 0.67×10^6 cells/mL gave a Chlorophyll *a* concentration of 850 µg/L. On an areal basis the maximum value for the Chlorophyll *a* component of the stratification in Lake Fidler was 60 mg/m^2, but it was usually much less than this and rarely exceeded 2.5 per cent of the total chlorophyll present. However, in the more pond-like Sulphide Pool, the Chlorophyll *a* component was much more significant, a range of 20-200 mg/m^2 being recorded, and on two occasions the Chlorophyll *a* component comprised 30 per cent of the total chlorophyll present.

In Lake Fidler the colourless sulphur bacteria *Beggiatoa* and cf. *Achromatium* are also present in extremely high numbers (up to 138,000 filaments/mL and 140,000 cells/mL respectively), but there is no published data on these organisms suitable for comparison.

Hence, the populations of the various organisms present in the microstratifications of Lake Fidler and Sulphide Pool are very large, and in terms of both cell concentrations and biomass on an areal basis, the lakes are amongst the most densely populated in the world.

4.5 PRODUCTIVITY OF THE MICROBIAL STRATIFICATIONS

Many determinations of carbon fixation have been made in stratified lakes to determine the relative contributions of algae, cyanobacteria, and photosynthetic bacteria. In most cases the photosynthetic bacterial contribution has been less than 10 per cent (e.g. Parkin and Brock, 1980a; Takahashi and Ichimura, 1968), but in some lakes it has been found to be much higher: Lawrence *et al.* (1978) reported a contribution of 46 per cent in Lake Waldsea; Culver and Brunskill (1969) 83 per cent in Fayetteville Green Lake; and Cohen *et al.* (1977) 91 per cent in Solar Lake. Absolute values for carbon fixation by natural populations of photosynthetic bacteria have been summarized by Wetzel (1975), who gave a range of 28 to 157 mg $C/m^2/day$, except for one value of 5,960 mg $C/m^2/day$ fixed by a "massive late summer population of *Chromatium*" in Smith Hole Lake, Indiana. Two other values, of 250 and 398 mg $C/m^2/day$, have been reported by Czeczuga (1968) and Lawrence *et al.* (1978) respectively. Rates of carbon fixation are available for several populations of photosynthetic bacteria in which *Chlorobium* is dominant: Czeczuga (1968) reported maximum rates of 20.5 and 180.4 mg $C/m^3/day$, in June 1966 and June 1967 respectively, from Lake Wadolek; Gorlenko *et al.* (1973) 160 mg $C/m^3/day$ in Reproe Lake; and Lawrence *et al.* (1978) and Culver and Brunskill (1969) reported maximum rates of 1,320 and 1,630 mg $C/m^3/day$ for Lake Waldsea and Fayetteville Green Lake respectively,two lakes in which photosynthetic bacteria make a major contribution to production (see above). Lindholm *et al.* (1984) in their report on Lake Holmsjon, in which Bacteriochlorophyll *d* values as high as 4,000 µg/L have been recorded, state simply that "photosynthetic activity in the dark green water was rather low, probably due to poor light conditons."

Only one carbon fixation experiment was carried out during this study, in Sulphide Pool on 12 December 1981 (see Section 3.5.2). Low pH values precluded accurate calculations of the amount of carbon fixed. For discussion purposes, a grossly underestimated value has been calculated at 40 mg $C/m^3/day$ for the photosynthetic bacteria, and 2,600 mg $C/m^3/day$ for the algae. The value of 2,600 mg $C/m^3/day$, particularly given that it is an underestimate, is surprisingly high. The algal population was almost entirely composed of *Scourfieldia caeca*, and it is obvious that very large amounts of inorganic carbon are being fixed by this organism in Sulphide Pool. The photosynthetic bacterial value of 40 mg $C/m^3/day$ is also surprisingly high. Although only a small faction of the algal fixation, it shows that there is also significant incorporation of inorganic carbon by the

photosynthetic bacteria of Sulphide Pool.

Similar fixation levels would be expected for the photosynthetic bacterial population of Lake Fidler. However, the percentage contribution of the photosynthetic bacteria to the overall fixation of carbon would probably be much higher in Lake Fidler, as the algal component of the microbial stratification is less prominent than that seen in Sulphide Pool.

The radiocarbon experiment carried out in Sulphide Pool indicates that significant amounts of inorganic carbon are fixed by both the algae and photosynthetic bacteria of the microbial stratification. Though the C^{14} method is fraught with difficulties, its application in Lake Fidler and Sulphide Pool, utilizing both organic and inorganic molecules, would no doubt yield interesting and valuable results. Firstly, the lakes are unusual in having very large numbers of algae present in the microbial stratifications, particularly in Sulphide Pool. Secondly, the photosynthetic bacteria of Lake Fidler would be expected to contribute significantly to production within the lake. And thirdly, heterotrophic uptake would be expected by several organisms, but particularly by the colourless sulphur bacteria in Lake Fidler.

4.6 UNDERWATER LIGHT AND SULPHIDE CONCENTRATIONS IN RELATION TO THE ORGANISMS OF THE MICROBIAL STRATIFICATIONS

The microbial stratifications of Lake Fidler and Sulphide Pool persistently receive less than 1 per cent of the light present at the surface of the lakes. At light levels of less than 1 per cent of surface irradiance, net photosynthesis by eukaryotes is conventionally assumed to be insignificant, and the depth of the 1 per cent light level is therefore assumed to be the lower limit of the euphotic zone (Westlake, 1980). This places the apparently productive layers of algae of the microbial stratifications of Lake Fidler and Sulphide Pool below the theoretical limit of the photic zone. Moreover, not only is light diminished markedly at the level of the algal layer, it is also severely limited in wavelength. Underwater light spectra at various depths in Sulphide Pool are shown in Figure 30. Below 1.30 metres the small amount of light that is present is mostly at wavelengths greater than 600 nm, light at wavelengths shorter than this having been absorbed by the organic matter dissolved in the waters of the lake (see below). At 1.40 metres the spectrum shows a marked trough

between 650-690 nm due to light absorption by *Scourfieldia caeca*. An *in vivo* scan of whole cells of *S. caeca* is superimposed on Figure 30, and a maximum absorption of the *in vivo* scan at 665-675 nm agrees well with the position of the trough.

Bindloss (1976) also found significant photosynthesis at depths greater than 1 per cent light penetration in Loch Leven. Clearly, there is justification for a reassessment of the arbitrary 1 per cent level. The flagellates of Lake Fidler and Sulphide Pool could perhaps be used as a "case-in-point" in this reassessment, their mode of nutrition, whether autotrophic or heterotrophic, being investigated in both the field and laboratory under various nutrient and light regimes.

With regard to the photosynthetic bacteria, light has been one of the two factors most often considered to be limiting production, the other being the supply of sulphide (e.g. Takahashi and Ichimura, 1970). However, Parkin and Brock (1980a) concluded that sulphide would seldom be a limiting factor, commenting that the sulphide concentration *per se* is unimportant if the rate of sulphide supply is sufficiently rapid, and that photosynthetic bacteria often grow in close association with the source of sulphide (e.g. sulphate-reducing bacteria) and might experience a higher sulphide concentration than that measured in a homogenous sample. Parkin and Brock concentrated on light, and plotted (for 16 lakes) the percentage contribution of photosynthetic bacteria to total primary production against the percentage of surface light reaching the depth of the photosynthetic bacterial populations. They found a more or less linear relationship between the two, and concluded that light intensity is likely to be the major factor limiting photosynthetic bacterial production in lakes. They then divided stratified hydrogen-sulphide containing lakes into three types, comprising those in which no light penetrates to the sulphide waters, e.g. lakes in which dense algal populations near the surface shade the deeper layers, those in which more than 5 per cent of surface light reaches the sulphide waters, e.g. those with a strong chemical stratification and clear surface waters, and those in which less than 0.5 per cent of surface light reaches the sulphide waters, e.g. dimictic or meromictic lakes with surface areas which are relatively small in relation to mean depths, so that the bottom of the thermocline approximates the maximum depth of light penetration.

With regard to underwater light, the two lakes fit the intermediate classification of Parkin and Brock, in that less than 5 per cent of surface light reaches the sulphide containing waters: in both Lake Fidler and

Purple and green photosynthetical bacteria possess pigments which absorb light at defined wavelengths, and the idea that differences in light quality at depth might be important in determining the type of photosynthetic bacteria present in a particular lake was put forward by Pfennig (1967). Field observations lent credence to the hypothesis, e.g. Truper and Genovese (1968) concluded that *Chlorobium* was dominant over *Chromatium* because its pigments absorbed particular wavelengths more efficiently, and culture studies showed that certain bacteria grow better than others under light regimes of particular wavelengths, e.g. Herbert and Tanner (1977).

Subsequent to their work on the effects of light intensity on photosynthetic bacterial production in lakes, Parkin and Brock turned their attention to the effects of light quality on bacterial growth, and studied in detail the spectral properties and bacterial populations of Lakes Mirror, Fish, and Knaack in Wisconsin (Parkin and Brock, 1980b). Their study is of particular relevance to the observations made herein, as Knaack Lake is similar in many ways to Lake Fidler and Sulphide Pool. Light penetration within it is relatively slight, as the waters are stained a yellow brown colour due to large amounts of dissolved humic and tannic materials, and the coloured waters absorb blue and green light to a greater extent than light of other colours. Absorption of these shorter wavelengths occurs to such an extent that at the depth of the photosynthetic bacteria only red light (ca. 700 nm) is available for photosynthesis. The waters of Lakes Mirror and Fish, on the other hand, are relatively clear, and green light (ca. 520-580 nm) is predominant at the depth of the photosynthetic bacterial populations.

Parkin and Brock observed that "only green sulphur bacteria were present in the study lake which transmits mainly red light (Knaack), and both purple and green sulphur bacteria were present in the two study lakes which transmit predominantly green light (Mirror and Fish)." Studies of enrichment cultures from the three lakes and photosynthetic responses of the natural populations indicated that "light quality is a major factor in determining the composition of phototrophic bacterial populations in some lakes."

The penetration of the spectrum of photosynthetically active radiation (400-740 nm) in Lake Fidler and Sulphide Pool is shown in Figures 29 and 30 respectively. The highly coloured waters, typical of South-West Tasmania, selectively absorb light of shorter wavelengths, and the higher

Sulphide Pool significantly less than 1 per cent reaches these depths. The data compiled by Parkin and Brock to plot their regression of underwater light against contribution to productivity suggest that the photosynthetic bacteria of Lake Fidler and Sulphide Pool would contribute 5 per cent or less to total productivity. The findings herein support this figure for Sulphide Pool, as large populations of algae also participate in the microstratification and fix significant amounts of inorganic carbon. However, the extremely large populations of photosynthetic bacteria in Lake Fidler could well contribute more than 5 per cent to total productivity, as fewer algae participate in the microbial stratification, and productivity in the surface waters is low. Given the data to hand, any estimate of the contribution of the photosynthetic bacteria to productivity in Lake Fidler could only be a guess, but it may well be sufficient to provide a major exception to the data gathered by Parkin and Brock to erect their classification. The determination of the per cent contribution of the photosynthetic bacteria to productivity within these lakes, particularly Lake Fidler, is thus an interesting project for the future.

With regard to dissolved sulphide in Lake Fidler and Sulphide Pool, that required by the photosynthetic bacteria appears to be supplied by diffusion from the deeper waters. During this study sulphide concentrations usually increased linearly with depth from the level of the redoxcline, and no obvious populations of sulphate-reducing bacteria were observed near the top of the monimolimnion. On two occasions the results for Lake Fidler suggested sulphide depletion by photosynthetic bacteria immediately below the redoxcline (Figs. 33 and 35). However, sulphide was still easily detected at the photosynthetic bacterial zone and it is unlikely on those, or any other, occasions that sulphide was a factor limiting production by photosynthetic bacteria in either Lake Fidler or Sulphide Pool.

The attenuation of light with depth in a particular water body is most often discussed only in terms of light intensity. However, there is another significant aspect to attenuation of light with depth; that concerning light quality. As light passes down the water column different wavelengths are attenuated to different degrees. The attenuation occurs in response to water colour and suspended material, and hence varies from lake to lake: organisms in a clear water, for instance, receive light closer to the blue end of the spectrum than organisms in a dystrophic water, as the dissolved organic material in the latter selectively absorbs blue light and has red light predominant at depth.

wavelengths (above 700 nm) are selectively absorbed by the water itself. At the depth of the photosynthetic bacterial populations of the two lakes only light of wavelengths above 600 nm is available for photosynthesis. From the work of Parkin and Brock, green sulphur bacteria would be expected to predominate in the brown waters of Lake Fidler and Sulphide Pool, and such is the case, with *Chlorobium* occurring at concentrations up to 40×10^6 cells/mL.

An *in vivo* absorption spectrum for whole cells of *Chlorobium* is superimposed on the spectra in Figure 29. Significant absorption was found above 600 nm with a peak absorption at 718 nm (the peak around 660 nm is due to absorption by degraded chlorophyll present because of cell lysis). Gloe *et al.* (1975) reported maximum red absorption peaks at wavelengths of 745-760 for green sulphur bacteria containing Bacteriochlorophyll *c*, 725-745 nm for those containing Bacteriochlorophyll *d*, and 715-725 nm for those containing Bacteriochlorophyll *e*, a new bacteriochlorophyll isolated from strains of brown-coloured green sulphur bacteria. At 718 nm the red absorption peak of *in vivo* scans for *Chlorobium* from Lake Fidler and Sulphide Pool is outside the range reported by Gloe *et al.* Parkin and Brock included *in vivo* scans in their paper on light quality and the red peak for their Bacteriochlorophyll *d* containing green sulphur bacteria in Knaack Lake (*Clathrochloris*, *Chlorobium* and *Pelodictyon*) was also outside the range of Gloe *et al.*, at around 710 nm. The precise forms of the *in vivo* absorption spectra of the green sulphur bacteria have been shown to depend on a large variety of parameters, including carbon source, source of reducing power, growth conditions, and age (Biebl and Drews, 1969). On the basis of the *in vivo* scans of cells from Lake Fidler, Sulphide Pool and Knaack Lake, perhaps light quality should be added to this list, as the light-harvesting mechanism of the photosynthetic bacteria appears to have adapted to suit the underwater light climate in these lakes.

4.7 PERMANENCE OF THE MEROMICTIC CONDITION IN LAKE FIDLER AND SULPHIDE POOL

The one discordant note of the project documented herein concerns the progressive degradation of the meromictic condition of Lake Fidler and Sulphide Pool.

Meromictic lakes are rare in Australia, and only five have been reported to date: West Basin Lake in Victoria (Timms, 1972), Lake Barrington in North-West Tasmania (Tyler and Buckney, 1974), and Lake Morrison, Lake Fidler and Sulphide Pool adjacent to the Gordon River in South-West Tasmania (King and Tyler, 1981b). The "Gordon Lakes" are the only ones for which a stratification of microorganisms has also been reported.

The origin and maintenance of the meromictic condition in Lake Morrison, Lake Fidler, and Sulphide Pool can be traced to a wedge of estuarine salt water penetrating the Gordon River at depth (King and Tyler, 1981b). A major hydro-electric power development upstream of the lakes has altered the flow regime of the river, such that the salt wedge no longer penetrates to an extent sufficient to maintain the meromictic condition (Bowling and Tyler, 1984). Lake Morrison has already become holomictic, and during this study the chemocline in Sulphide Pool dropped 0.50 metres, and that in Lake Fidler 0.20 metres. Unless river flows are manipulated to allow salt water to penetrate sufficiently far upstream, the mixolimnion of Lake Fidler and Sulphide Pool will continue to deepen, and these lakes will also become holomictic. Proposals concerning such manipulations in flows have been put to the power development authority (Tyler, pers. comm.), but given the time-frame involved, it is unlikely that the trend towards holomixis will be arrested, and probable that the meromictic nature of the lakes will be lost. In the relatively shallow Sulphide Pool, the loss of the unique microstratification of organisms and the meromictic condition *per se* will be concomitant, as light penetrates almost to the lake bottom. In the deeper Lake Fidler, the microstratification will be lost before the lake becomes holomictic, as the slowly-sinking redoxcline will pass below the depth to which light penetrates well before the lake is completely mixed. A different array of microorganisms will no doubt be present about the redoxcline when this occurs, perhaps dominated by colourless sulphur bacteria.

4.8 CONCLUDING REMARKS

At the beginning of this study Lake Fidler and Sulphide Pool were known to be superb examples of the meromictic condition, and to contain an interesting assemblage of microorganisms at depth. The work documented herein has identified the organisms present in the lakes, determined their biomass, and elucidated their precise distribution with respect to the prevailing physical and chemical conditions. In doing these things it has also enhanced the reputation of the lakes as unusual and interesting limnological sites. Of particular note are the following:

(1) The lakes contain perhaps the most graphic examples in the world of algal/bacterial layering at depth, comprising flagellate algae, colourless sulphur bacteria, photosynthetic bacteria, and an ectosymbiotic consortium.

(2) The layering, as determined by a sampler constructed specifically for the purpose, is particularly precise and stable.

(3) The flagellate alga *Scourfieldia caeca* is a prominent component of the layering. This organism, previously known only from temporary ponds in Europe, is a permanent member of the microbial array, at concentrations up to 860,000 cells/mL.

(4) The green photosynthetic bacterium *Chlorobium* is present in concentrations as high as those recorded anywhere in the world. Maximum concentrations are recorded immediately below the redoxcline, but large numbers of viable cells are also present in oxygenated waters above it.

(5) The enigmatic ectosymbiotic consortium *"Chlorochromatium aggregatum"* is also present in high numbers. It is the only "organism" of the microbial arrays to display any degree of movement within the water column, and can be found either above or below the redoxcline.

(6) The photosynthetic organisms of the microbial arrays photosynthesise at extremely low levels of red light.

(7) The lakes are particularly well endowed with Chrysophytes, especially members of the Mallomonadaceae. Fourteen species (half the number recorded for the whole of Australia) are to be found in them, including two new taxa, one of which is yet to be found outside Sulphide Pool.

It would be pleasing if the scientific work on Lake Fidler and Sulphide Pool continued, as these unique environments still have much to

offer. A study of nutrient cycling and the utilization of carbon, for instance, would yield interesting results. Hopefully, the work documented herein will help to maintain an interest in the lakes, and they will both be investigated further before they become holomictic.

Roger Broome
1st May 1984.

5. REFERENCES

ANON., (1971). Standard methods for examination of water and wastewater. 13th Edn. American Public Health Association, N.Y.

ANTON, A. & DUTHIE, H.C. (1981). Use of cluster analysis in the systematics of the algal genus *Cryptomonas*. CAN. J. BOT., 59, 6, 992-1002.

AZAM, F., FENCHEL, T., FIELD, J.G., GRAY, J.S., MEYER-REIL, L.A. & THINGSTAD, F. (1983). The ecological role of water-column microbes in the sea. MAR. ECOL. PROG. SER., 10, 257-263.

BAKER, A.L. (1970). An inexpensive microsampler. LIMNOL. OCEANOGR., 15, 1, 158-160.

BAKER, A.L., BAKER, K.K. & TYLER, P.A. The microanatomy of meromixis - a family of microsamplers (In prep.).

BAKER, A.L., BAKER, K.K. & TYLER, P.A. Close interval sampling of migrating *Chaoborus* larvae across the chemocline of meromictic Lake Fidler, Tasmania. ARCHIV FUR HYDROBIOLOGIE (In prep.).

BELCHER, J.H. (1964). Further notes on *Scourfieldia caeca*. BR. PHYCOL. BULL., 2, 371-373.

BELCHER, J.H. & SWALE, E.M.F. (1963). Some new and uncommon British Volvocales II. BR. PHYCOL. BULL., 2, 210-218.

BIEBL, H., & DREWS, G. (1969). Das *in vivo* spectrum als taxonomisches merkmal bei untersuchungen zur verbreitung von Athiorhodaceae. ZENTRALBL. BAKTERIOL. PARASITENKD. INFEKTIONSKR. HYG. ABT., 2, 123, 425.

BINDLOSS, M.E. (1976). The light climate of Loch Leven, a shallow Scottish lake, in relation to primary production by phytoplankton. FRESHWATER BIOL., 6, 501-518.

BLAKAR, I.A. (1979). A close-interval water sampler with minimal disturbance properties. LIMNOL. OCEANOGR., 24, 5, 983-988.

BLAUBOER, C.I. (1982). The phytoplankton species composition and the seasonal periodicity in Lake Vechten from 1956 to 1979. HYDRO-BIOLOGIA, 95, 25-36.

BOURRELLY, P. (1966). Les Algues d'Eau Douce: Les Algues Vertes. Editions N. Boubee & Cie, Paris.

BOURRELLY, P. (1968). Les Algues d'Eau Douce: Les Algues Jaune et Brune. Editions N. Boubee & Cie, Paris.

BOWLING, L.C. (1981). Limnological studies on the meromictic lakes of the Lower Gordon River. Hons. Thesis. Univ. of Tasmania.

BOWLING, L.C. & TYLER, P.A. (1984). Endangered lakes of scientific and cultural value in the world heritage area of South-West Tasmania. BIOL. CONS. (In press).

BROCK, T.D., BROCK, M.L., BOTT, T.L. & EDWARDS, M.R. (1971). Microbial life at 90°C: the sulfur bacteria of Boulder Spring. J. BACTERIOL., 107, 303-314.

BUCHANAN, R.E. & GIBBONS, N.E. (Eds.) (1974). Bergey's Manual of Determinative Bacteriology. Eighth Ed. The Williams & Wilkins Co. Baltimore.

BUCKNEY, R.T. & TYLER, P.A. (1973a). Chemistry of some sedgeland waters, Lake Pedder, south-west Tasmania. AUST. J. MAR. FRESHWATER RES., 24, 267-273.

BUCKNEY, R.T. & TYLER, P.A. (1973b). Chemistry of Tasmanian inland waters. INT. REV. GES. HYDROBIOL., 58, 1, 61-78.

BUDER, J. (1914). *Chloronium mirabile*. BERICHTE DER DEUTSCHEN BOTANISCHEN GESELLSCHAFT, 31, 80-97.

CALDWELL, D.E. & TIEDJE, J.M. (1975). The structure of anaerobic bacterial communities in the hypolimnion of several Michigan lakes. CAN. J. MICROBIOL., 21, 3, 377-385.

CHEBOTAREV, E.N., GORLENKO, V.M. & KACHALKIN, V.I. (1973). Microbiological hydrogen sulphide formation in Lake Repnoe (Slavyansk Lakes). MIKROBIOLOGIYA, 42, 3, 475-480.

CHENG, D.M.H. & TYLER, P.A. (1973). Lakes Sorell and Crescent - a Tasmanian paradox. INT. REV. GES. HYDROBIOL., 58, 3, 307-343.

COHEN, Y., KRUMBEIN, W.E. & SHILO, M. (1977). Solar Lake (Sinai) 2. Distribution of photosynthetic microorganisms and primary production. LIMNOL. OCEANOGR. 22, 609-620.

COMPERE, P. (1974). *Mallomonas bronchartiana*, Chrysophyceae nouvelle du lac Tchad. BULL. JARD. BOT. NAT. BELG./BULL. NAT. PLANTENTUIN BELG., 44, 61-63.

CONRAD, W. (1933). Revision du genre *Mallomonas* Perty (1851) including *Pseudo-Mallomonas* Chodat (1920). MEM. ROG. D'HISTOIRE NAT. BELGIQUE, 56.

CROOME, R.L. (1980). Lake Hume. In Williams, W.D. (Ed.). An ecological basis for water resource management. Aust. Nat. Univ. Press, Canberra, Australia.

CROOME, R.L., DURRSCHMIDT, M. & TYLER, P.A. (1984). A light and electron microscopical investigation of *Mallomonas splendens* (West) Playfair. NOVA HEDWIGIA (Submitted).

CROOME, R.L. & TYLER, P.A. (1972). Physical and chemical limnology of Lake Leake and Tooms Lake, Tasmania. ARCH. HYDROBIOL., 70, 341-354.

CROOME, R.L. & TYLER, P.A. (1973). Plankton populations of Lake Leake and Tooms Lake - oligotrophic Tasmanian lakes. BR. PHYCOL. J., 8, 239-247.

CROOME, R.L. & TYLER, P.A. (1983a). *Mallomonas plumosa* (Chrysophyceae), a new species from Australia. BR. PHYCOL. J., 18, 151-158.

CROOME, R.L. & TYLER, P.A. (1983b). *Mallomonopsis tasmanica* sp. nov. (Chrysophyceae) and *Mallomonopsis elliptica* Matvienko from Australia. BR. PHYCOL. J., 18, 357-365.

CROOME, R.L. & TYLER, P.A. (1983c). *Mallomonas morrisonensis* (Chrysophyceae), a new species from Australia. BR. PHYCOL. J., 18, 383-389.

CROOME, R.L. & TYLER, P.A. (1984a). Microbial microstratification and crepuscular photosynthesis in meromictic Tasmanian lakes. V.I.V.L. (In press).

CROOME, R.L. & TYLER, P.A. (1984b). The micro-anatomy and ecology of *Scourfieldia caeca* (Korsh.) Belcher et Swale in two meromictic lakes in Tasmania. BR. PHYCOL. J. (Submitted).

CROOME, R.L. & TYLER, P.A. (1984c). The micro-anatomy and ecology of "*Chlorochromatium aggregatum*" in two meromictic lakes in Tasmania. J. GEN. MICROBIOL. (Submitted).

CROOME, R.L. & TYLER, P.A. (1984d). Australian Mallomonadaceae (Chrysophyceae). NORDIC J. BOTANY (Submitted).

CROOME, R.L. & TYLER, P.A. (1984e). A re-examination of Playfair's *Synura australiensis* and *Synura granulosa*. NORDIC J. BOTANY (Submitted).

CULVER, D.A. & BRUNSKILL, G.J. (1969). Fayetteville Green Lake, New York. 5. Studies of primary production and zooplankton in a meromictic marl lake. LIMNOL. OCEANOGR., 14, 862-873.

CZEZUGA, B. (1968). Primary production of the green hydrosulphuric bacteria, *Chlorobium limicola* Nads. (Chlorobacteriaceae). PHOTOSYNTHETICA, 2, 1, 11-15.

DE BOER, W.E., LA RIVIERE, J.W.M. & SCHMIDT, K. (1971). Some properties of *Achromatium oxaliferum*. ANTONIE VAN LEEUWENHOEK. 37, 553-563.

DUBININA, G.A., GORLENKO, V.M. & SULEIMANOV, Y.I. (1973). A study of microorganisms involved in the circulation of manganese, iron, and sulfur in meromictic Lake Gel-Gel. MIKROBIOLOGIYA, 42, 5, 918-924.

DUBININA, G.A. & KUZNETSOV, S.I. (1976). The ecological and morphological characteristics of microorganisms in Lesnaya Lamba (Karelia). INT. REV. GES HYDROBIOL., 61, 1, 1-19.

DURRSCHMIDT, M. (1980). Studies on the Chrysophyceae from Rio Cruces, Prov. Valdivia, South Chile by scanning and transmission microscopy. NOVA HEDWIGIA, 33, 353-388.

DURRSCHMIDT, M. (1982). Studies on the Chrysophyceae from South Chilean inland waters by means of scanning and transmission electron microscopy II. ARCH. HYDROBIOL., SUPP-BD., 63, 121-163.

DURRSCHMIDT, M. (1983). A taxonomic study of the *Mallomonas mangofera* group (Mallomonadaceae, Chrysophyceae), including the description of four new taxa. PL. SYST. EVOL., 143, 175-196.

DURRSCHMIDT, M. & CROOME, R.L. (1984). Contribution to the knowledge of Mallomonadaceae (Chrysophyceae) from Malaysia and Australia. NORDIC J. BOTANY (Submitted).

DUTHIE, H.C. (1979). Limnology of subarctic Canadian lakes and some effects of impoundment. ARCT. ALP. RES., 11, 145-158.

FENCHEL, T. (1982). Ecology of heterotrophic microflagellates. I. Some important forms and their functional morphology. MAR. ECOL. PROG. SER., 8, 211-223.

GLOE, A., PFENNIG, N., BROCKMANN, H. & TROWITZSCH, W. (1975). A new bacteriochlorophyll from brown-coloured Chlorobiaceae. ARCH. MICROBIOL., 102, 103-109.

GOLTERMAN, H.L. (Ed.). (1969). Methods for chemical analysis of fresh waters IBP Handbook No. 8. Blackwell Scientific Publications, Oxford Co., Edin.

GOLTERMAN, H.L., CLYMO, R.S. & OHNSTAD, M.A.M. (1978). Methods for physical and chemical analysis of fresh waters. IBP Handbook No. 8. Blackwell Scientific Publications, Oxford, Melb.

GORLENKO, V.M. & CHETABOREV, E.N. (1981). Microbiological processes in the meromictic Lake Sakovo. MICROBIOLOGY, 50, 1, 98-102.

GORLENKO, V.M., CHEBOTAREV, E.N. & KACHALKIN, V.I. (1973). Microbiological processes of oxidation of hydrogen sulphide in the Repnoe Lake (Slavonic Lakes). MICROBIOLOGY, 42, 4, 643-646.

GORLENKO, V.M., CHEBOTAREV, E.N. & KACHALKIN, V.I. (1974a). Microbial oxidation of hydrogen sulphide in Lake Veisovo (Slavyansk Lake). MICROBIOLOGY, 43, 1-3, 450-453.

GORLENKO, V.M., CHEBOTAREV, E.N. & KACHALKIN, V.I. (1974b). Participation of microorganisms in the circulation of sulphur in Pomyaretskoe Lake. MICROBIOLOGY, 43, 4-6, 772-776.

GORLENKO, V.M., DUBININA, G.A. & KUZNETSOV, C.I. (1977). The ecology of aquatic microorganisms. Science Publn. Acad. Sciences, U.S.S.R. Moscow.

GORLENKO, V.M. & KUZNETSOV, S.I. (1971). Vertical distribution of photosynthetic bacteria in the Konon"er Lake of the Mari U.S.S.R. MICROBIOLOGY, 40, 651-652.

GORLENKO, W.M. & LEBEVA, G.V. (1971). Vertical distribution of photosynthetic bacteria in the Konon'er Lake in the Mari, U.S.S.R. MICROBIOLOGY, 40, 651-652.

GORLENKO, V.M. & LOKK, S.I. (1979). Vertical distribution and special composition of microorganisms from some stratified Estonian lakes. MIKROBIOLOGIYA, 48, 351-359.

GUERRERO, R., MONTESINOS, E., ESTEVE, I. & ABELLA, C. (1980). Physiological adaptation and growth of purple and green sulfur bacteria in a meromictic lake (Vila) as compared to a holomictic lake (Siso). In DOKULIL, M., METZ, H. & JENSON, D. (Eds.). Developments in Hydrobiology, 3, 161-171. Dr. W. Junk, The Hague.

HAFFNER, G.D., HARRIS, G.P. & JARAI, M.K. (1980). Physical variability and phytoplankton communities. III. Vertical structure in phytoplankton populations. ARCH. HYDROBIOL., 89, 3, 363-381.

HAMMER, U.T., HAYNES, R.C., LAWRENCE, J.R. & SWIFT, M.C. (1978). Meromixis in Waldsea Lake, Saskatchewan. V.I.V.L., 20, 192-200.

HARRIS, K. (1967). Variability in *Mallomonas*. J. GEN. MICROBIOL., 46, 185-191.

HERBERT, R.A. & TANNER, A.C. (1977). The isolation and some characteristics of photosynthetic bacteria (Chromatiaceae and Chlorobiaceae) from Antarctic marine sediments. J. APPL. BAC., 43, 437-445.

HUBER-PESTALOZZI, G. (1941). Das Phytoplankton des Susswassers. Die Binnengewasser, 16, 2, 1. Stuttgart.

HUBER-PESTALOZZI, G. (1961). Das Phytoplankton des Susswassers. Die Binnengewasser, 16, 5. Stuttgart.

ILMAVIRTA, V. (1980). Phytoplankton in 35 Finnish brown-water lakes of different trophic status. Developments in Hydrobiology, 3 (Dokulil, M. *et al.*, Eds.). Dr. W. Junk, The Hague.

ILMAVIRTA, V. (1983). The role of flagellated phytoplankton in chains of small brown-water lakes in southern Finland. ANN. BOT. FENNICI, 20, 187-195.

ILMAVIRTA, V. (1984). The ecology of flagellated phytoplankton in brown-water lakes: the significance of their vertical migrations. V.I.V.L. (In press).

JEFFREY, S.W. & VESK, M. (1977). Effect of blue-green light on photosynthetic pigments and chloroplast structure in the marine diatom *Stephanopyxis turris*. J. PHYCOL., 13, 271-279.

JENSEN, A., AASMUNDRUD, O. & EIMHJELLEN, K.E. (1964). Chlorophylls of photosynthetic bacteria. BIOCHIM. BIOPHYS. ACTA, 88, 466-479.

JONES, J.G. (1979). A guide to methods for estimating microbial numbers and biomass in fresh water. Freshwater Biological Association Scientific Publication No. 39.

JORGENSEN, B.B. (1982). Ecology of the bacteria of the sulphur cycle with special reference to anoxic-oxic interface environments. PHIL. TRANS. R. SOC. (B), 298, 1093, 543-561.

KERR, J. (1960). The spectrophotometric determination of microgram amounts of calcium. ANALYST (LONDON), 85, 867-870.

KING, R.D. & TYLER, P.A. (1981a). Limnology of Perched Lake, South-west Tasmania. AUST. J. MAR. FRESHWATER RES., 32, 501-515.

KING, R.D. & TYLER, P.A. (1981b). Meromictic lakes of South-west Tasmania. AUST. J. MAR. FRESHWATER RES., 32, 741-756.

KING, R.D. & TYLER, P.A. (1982a). Lake Fidler, a meromictic lake in Tasmania. ARCH. HYDROBIOL., 93, 4, 393-422.

KING, R.D. & TYLER, P.A. (1982b). Downstream effects of the Gordon River power development, South-west Tasmania. AUST. J. MAR. FRESHWATER RES., 33, 431-442.

KING, R.D. & TYLER, P.A. (1983). Sulphide Pool and Lake Morrison, meromictic lakes of south-west Tasmania. ARCH. HYDROBIOL., 96, 139-163.

KIRK, J.T.O. (1976). Yellow substance (Gelbstoff) and its contribution to the attenuation of photosynthetically active radiation in some inland and coastal South-Eastern Australian waters. AUST. J. MAR. FRESHWATER RES., 27, 61-71.

KORSHIKOV, A.A. (1916). Materialy k flore vodorostei Rossii. *Cardiomonas caeca* nov. gen. et sp. iz gruppy zelenykh Flagellata. J. MICROBIOL., PETROGRAD, 3, 61-68, 233-234.

KRISTIANSEN, J. (1975). Chrysophyceae from Alberta and British Columbia. SYESIS, 8, 97-108.

KRISTIANSEN, J. (1980). A checklist of Danish freshwater Chrysophyceae. Institute of Plant Anatomy and Cytology, University of Copenhagen.

KRISTIANSEN, J. (1981). Distribution problems in the Synuraceae (Chrysophyceae). V.I.V.L., 21, 1444-1448.

KRISTIANSEN, J. (1984). Occurrence of scale-bearing Chrysophyceae in a eutrophic Danish lake. V.I.V.L. (In press).

KUENEN, J.G. (1975). Colourless sulfur bacteria and their role in the sulfur cycle. PLANT & SOIL, 43, 49-76.

LA RIVIERE, J.W.M. & SCHMIDT, K. (1981). Morphologically conspicuous sulfur-oxidizing eubacteria. In STARR, M.P., STOLP, H., TRUPER, H.G., BALOWS, A. & SCHLEGEL, H.G. (Eds.). The Prokaryotes. Vol. I. Springer-Verlag. Berlin, N.Y.

LAUTERBORN, R. (1906). Zur kenntnis der sapropelischen flora. ALLGEMEINE BOTANISCHE ZEITSCHRIFT, 12, 196-197.

LAUTERBORN, R. (1916). Die sapropelische lebewelt. Ein beitrag zur biologie des faulschlammes naturlicher gewasser. VERH. NATURH. MEDIZ. VER. HEIDELB., 13, 395-481.

LAWRENCE, J.R., HAYNES, R.C. & HAMMER, U.T. (1978). Contribution of photosynthetic green sulphur bacteria to total primary productivity in a meromictic saline lake. V.I.V.L., 20, 201-207.

LEE, R.E. (1980). Phycology. Cambridge University Press, Cambridge, N.Y.

LEEDALE, G.F. (1975). Envelope formation and structure in the Euglenoid genus *Trachelomonas*. BR. PHYCOL. J., 10, 17-41.

LINDHOLM, T., WEPPLING, K. & JENSEN, S. (1984). Stratification and primary production in a small brackish lake studied by close-interval siphon sampling. V.I.V.L. (In press).

LING, H.U. & TYLER, P.A. (1984). Freshwater algae exclusive of diatoms of the Alligator Rivers Region, Northern Territory. Supervising Scientist for the Alligator Rivers Region. Res. Reps. (In press).

LUND, J.W.G. (1942). Contributions to our knowledge of British algae VIII. J. BOT., LOND., 80, 57-73.

LUND, J.W.G., KIPLING, C. & Le CREN, E.D. (1958). The inverted microscope method of estimating algal numbers and the statistical basis of estimations by counting. HYDROBIOLOGIA, 11, 2, 143-170.

McLEOD, J.A. (1975). The freshwater algae of South-eastern Queensland. Ph.D. Thesis, University of Queensland.

MANTON, I. (1975). Observations of the microanatomy of *Scourfieldia marina* Throndsen and *Scourfieldia caeca* (Korsh.) Belcher & Swale. ARCH. PROTISTENK, 117, 358-368.

MARCHANT, H.J. & THOMAS, D.P. (1983). Polylysine as an adhesive for the attachment of nanoplankton to substrates for electron microscopy. J. MICROS., 131, 127-129.

MELKONIAN, M. & PREISIG, H.R. (1982). Twist of central pair microtubules in the flagellum of the green flagellate *Scourfieldia caeca*. CELL BIOL. INT. REPTS., 6, 269-277.

MOESTRUP, O. (1982). Flagellar structure in algae: a review, with new observations particularly on the Chrysophyceae, Phaeophyceae (Fucophyceae), Euglenophyceae, and *Reckertia*. PHYCOLOGIA, 21, 4, 427-528.

MORITA, R.Y. & STAVE, P.W. (1963). Electron micrograph of an ultrathin section of *Beggiatoa*. J. BACTERIOL., 85, 940-942.

MUNAWAR, M. & MUNAWAR, I.F. (1975). Abundance and significance of phytoflagellates and nannoplankton in the St. Lawrence Great Lakes. VERH. INT. VER. THEOR. ANGEW. LIMNOL., 19, 705-723.

NADSON, G.A. & VISLOUKH, S.M. (1923). Formation and life of the gigantic bacterium *Achromatium oxaliferum* Schew. BULL. JARD. BOT. URSS., (Suppl. 1), 22, 1-37.

NICHOLLS, K.H. (1982). *Mallomonas* species (Chrysophyceae) from Ontario, Canada including descriptions of two new species. NOVA HEDWIGIA, 36, 1, 89-124.

NORRIS, R.E. (1980). Prasinophytes. In Cox, E.R. (Ed.). Developments in Marine Biology. Vol. 2. Phytoflagellates. Elsevier, New York.

OLSON, J.M. & STANTON, E.K. (1966). Absorption and fluorescence spectra of bacterial chlorophylls *in situ*. In Vernon, L.P. & Seely, G.R. (Eds.). The Chlorophylls. Academic Press, N.Y. & London.

PARKER, B.C., LEEPER, G. & HURNI, W. (1968). Sampler for studies of thin horizontal layers. LIMNOL. OCEANOGR., 13, 172-175.

PARKIN, T.B. & BROCK, T.D. (1980a). Photosynthetic bacterial production in lakes: the effects of light intensity. LIMNOL. OCEANOGR., 25, 711-718.

PARKIN, T.B. & BROCK, T.D. (1980b). The effects of light quality on the growth of phototrophic bacteria in lakes. ARCH. MICROBIOL., 125, 19-27.

PARKIN, T.B. & BROCK, T.D. (1981). Photosynthetic bacterial production and carbon mineralization in a meromictic lake. ARCH. HYDROBIOL., 91, 3, 366-382.

PERFILIEV, B.V. (1914). On the theory of symbiosis of *Chlorochromatium aggregatum* Lauterb. (*Chloronium mirable* Buder) and *Cylindrogloea bacterifera* nov. gen. nov. sp. ZHURNAL MIKROBIOLOGII PETROGRAD, 1, 3-5, 222-227.

PETERFI, L.S. (1966). Studies on the Rumanian Chrysophyceae I. NOVA HEDWIGIA, 13, 117-137.

PETERFI, L.S. & MOMEU, L. (1976). Romanian *Mallomonas* species studied in light and electron microscopes. NOVA HEDWIGIA, 27, 353-392.

PFENNIG, N. (1967). Photosynthetic bacteria. ANN. REV. MICROBIOL., 21, 285-324.

PFENNIG, N. (1978). General physiology and ecology of photosynthetic bacteria. In Clayton, R.K. & Sistrom, W.R. (Eds.). The photosynthetic bacteria. Plenum Press, N.Y.

PFENNIG, N. (1980). Syntrophic mixed cultures and symbiotic consortia with phototrophic bacteria: a review. In Gottschalk *et al.* (Eds.), Anaerobes and anaerobic infections. Gustav Fischer Verlag, Stuttgart, New York.

PFENNIG, N. & LIPPERT, K.D. (1966). Uber das vitamin B_{12} - bedurfnis phototropher schwefelbakterien. ARCH. MIKROBIOL., 55, 245-256.

PHILIPOSE, M.T. (1953). Contributions to our knowledge of Indian algae I. Chrysophyceae - on the occurrence of *Mallomonas* Perty, *Synura* Ehr., and *Dinobryon* Ehr. in India. PROC. INDIAN ACAD. ACI., 37, 331-338.

PLAYFAIR, G.I. (1912). Plankton of the Sydney water supply. PROC. LINN. SOC. N.S.W., 37, 512-552.

PLAYFAIR, G.I. (1915). Freshwater algae of the Lismore district: with an appendix on the algal fungi and schizomycetes. PROC. LINN. SOC. N.S.W., 40, 310-362.

PLAYFAIR, G.I. (1921). Australian freshwater flagellates. PROC. LINN. SOC. N.S.W., 46, 99-146.

PREISIG, H.R. & HIBBERD, D.J. (1982a). Ultrastructure and taxonomy of *Paraphysomonas* (Chrysophyceae) and related genera I. NORD. J. BOT., 2, 397-420.

PREISIG, H.R. & HIBBERD, D.J. (1982b). Ultrastructure and taxonomy of *Paraphysomonas* (Chrysophyceae) and related genera II. NORD. J. BOT., 2, 601-638.

PRINGSHEIM, E.G. (1953). Observations on some species of *Trachelomonas* grown in culture. NEW PHYTOL., 52, 238-266.

PRINGSHEIM, E.G. (1967). Die mixotrophie von *Beggiatoa*. ARCHIV. FUR MIKROBIOLOGIE, 59, 247-254.

PROWSE, G.A. (1962). Further Malayan freshwater flagellata. GARDENS BULL. SINGAPORE, 19, 105-145.

RITTENBERG, S.C. (1969). The roles of exogenous organic matter in the physiology of chemolithotrophic bacteria. ADV. MICROBIAL PHYSIOL., 3, 159-196.

ROUND, F.E. (1981). The ecology of algae. Cambridge Univ. Press, Cambridge, London, N.Y.

RUTTNER, F. & SAUBERGER, F. (1938). Durschsichtigkeit des Wassers und Planktonschichtung. INT. REV. GES. HYDROBIOL., 37, 405-419.

SCOURFIELD, D.J. (1944). The nannoplankton of bomb-crater pools in Epping Forest. ESSEX NAT., 27, 231-241.

SOROKIN, Ju. I. & DONATO, N. (1975). On the carbon and sulphur metabolism of the meromictic Lake Faro (Sicily). HYDROBIOLOGIA, 47, 2, 241-252.

STANIER, R.Y., DOUDOROFF, M. & ADELBERG, E.A. (1971). General Microbiology (3rd Ed.). Macmillan, London.

STANIER, R.Y., PFENNIG, N. & TRUPER, H.G. (1981). Introduction to the phototrophic prokaryotes. In Starr, M.P. *et al.* (Eds.). The prokaryotes, Vol. 1. Springer-Verlag, Berlin, N.Y.

STANIER, R.Y. & SMITH, J.H.C. (1960). The chlorophylls of green bacteria. BIOCHIM. BIOPHYS. ACTA., 41, 478-484.

STEEMAN NIELSEN, E. (1952). The use of radio-active carbon (C^{14}) for measuring organic production in the sea. J. CONS. PERM. INT. EXPLOR. MER., 18, 117-140.

STEENBERGEN, C.L.M. (1982). Contribution of photosynthetic sulphur bacteria to primary production in Lake Vechten. HYDROBIOLOGIA, 95, 59-64.

STEENBERGEN, C.L.M. & KORTHALS, H.J. (1982). Distribution of phototrophic microorganisms in the anaerobic and microaerophilic strata of Lake Vechten (The Netherlands). Pigment analysis and role in primary production. LIMNOL. OCEANOGR., 27, 883-895.

STOEMER, E.F. & SICKO-GOAD, L. (1977). A new distribution record for *Hyenomonas roseola* Stein (Prymnesiophyceae, Coccolithophoraceae) and *Spiniferomonas trioralis* Takahashi (Chrysophyceae, Synuracea) in the Laurentian Great Lakes. PHYCOLOGIA, 16, 4, 355-358.

STUMM, W. (1967). Redox potential as an environmental parameter; conceptual significance and operational limitation. In Advances in Water Pollution Research, pp. 283-298. Baltimore, Port City Press.

TAKAHASHI, E. (1959). Studies on genera *Mallomonas, Synura* and other plankton in freshwater with the electron microscope I. BULL. YAMAGATA UNIV., AGR. SCI., 3, 1, 117-151.

TAKAHASHI, E. (1973). Studies on genera *Mallomonas* and *Synura*, and other plankton in freshwater with the electron microscope VII. New genus *Spiniferomonas* of the Synuraceae (Chrysophyceae). BOT. MAG. TOKYO, 86, 75-88.

TAKAHASHI, E. (1975). The fine structure of the scales and flagella of the Chrysophyta. In Tokida, J. & Hirose, H. (Eds.). Advance of Phycology in Japan. Dr. W. Junk, The Hague.

TAKAHASHI, E. (1978). Electron microscopical studies on the Synuraceae (Chrysophyceae) in Japan. Tokai Univ. Press, Tokyo.

TAKAHASHI, M. & ICHIMURA, S. (1968). Vertical distribution and organic matter production of photosynthetic sulphur bacteria in Japanese lakes. LIMNOL. OCEANOGR., 13, 644-655.

TAKAHASHI, M. & ICHIMURA, S. (1970). Photosynthetic properties and growth of photosynthetic sulphur bacteria in lakes. LIMNOL. OCEANOGR., 15, 929-944.

TALLING, J.F. (1981). The development of attenuance depth-profiling to follow the changing distribution of phytoplankton and other particulate material in a productive English lake. ARCH. HYDROBIOL., 93, 1, 1-20.

THOMASSON, K. (1963). Araucanian lakes. ACTA PHYTOGEOGRAPHICA SUECICA, 47.

THOMASSON, K. (1973). Notes on the plankton of some Sydney reservoirs with descriptions of two interesting desmids. CONTRIB. N.S.W. NAT. HERB., 4, 6, 384-394.

THRONDSEN, J. (1969). Flagellates of Norwegian coastal waters. NYTT. MAG. BOT., 16, 183-185.

TIMMS, B.V. (1972). A meromictic lake in Australia. LIMNOL. OCEANOGR., 17, 918-922.

TRUPER, H.G. & GENOVESE, S. (1968). Characterization of photosynthetic sulphur bacteria causing red water in Lake Faro (Messina, Sicily). LIMNOL. OCEANOGR., 13, 225-232.

TRUPER, H.G. & PFENNIG, N. (1971). Family of phototrophic green sulfur bacteria: *Chlorobiaceae* Copeland, the correct family name; rejection of *Chlorobacterium* Lauterborn; and the taxonomic situation of the consortium-forming species. Request for an opinion. INT. J. SYST. BACT., 21, 8-10.

TRUPER, H.G. & PFENNIG, N. (1978). Taxonomy of the Rhodospirillales. In Clayton, R.K. & Sistrom, W.R. (Eds.). The photosynthetic bacteria. Plenum Press, N.Y.

TYLER, P.A. & BUCKNEY, R.T. (1974). Stratification and biogenic meromixis in Tasmanian reservoirs. AUST. J. MAR. FRESHWATER RES., 25, 299-313.

VAN NIEL, C.B. (1963). A brief summary of the photosynthetic bacteria, p.459-467. In Gest, H. *et al.* (Eds.). Bacterial photosynthesis. Antioch.

VAN NIEL, C.B. (1971). Techniques for the enrichment, isolation, and maintenance of the photosynthetic bacteria. In A. San Pietro (Ed.). Methods in Enzymology. Vol. XXIII. Photosynthesis. Part A. Academic Press, N.Y. & London.

VESK, M. & JEFFREY, S.W. (1977). Effect of blue-green light on photosynthetic pigments and chloroplast structure in unicellular marine algae from six classes. J. PHYCOL., 13, 280-288.

VIYAKORNVILAS, K. (1974). Some algae in Lakes Hume and Mulwala. Hons. Thesis, University of Tasmania.

VOLLENWEIDER, R.A. (Ed.). (1964). A manual on methods for measuring primary production in aquatic environments. I.B.P. Handbook No. 12. 2nd Ed. Blackwell Sc. Publ. Oxford, Melbourne.

WALKER, T.D. & TYLER, P.A. (1984). Chemical characteristics and nutrient status of billabongs of the Alligator Rivers Region, Northern Territory. Supervising Scientist for the Alligator Rivers Region Res. Reps. (In press).

WEST, G.S. (1909). The algae of the Yan Yean Reservoir, Victoria: a biological and ecological study. J. LINN. SOC. (BOT.), 39, 1-88.

WEST, G.S. (1912). Algological notes X-XII. J. BOT., LOND., 50, 321-331.

WEST, L,K., WALNE, P .L. & BENTLEY, J. (1980). *Trachelomonas hispida* var. *coronata* (Euglenophyceae): III Envelope elemental composition and mineralization. J. PHYCOL., 16, 582-591.

WESTLAKE, D.F. (Co-ordinator) (1980). Primary production. In Le Cren, E.D. & Lowe-McConnell, R.H. (Eds.). The functioning of freshwater ecosystems. Cambridge Univ. Press, Cambridge, New York.

WETZEL, R.G. (1975). Limnology. W.B. Saunders Co., Philadelphia.

WHITFIELD, M. (1969). Eh as an operational parameter in estuarine studies. LIMNOL. OCEANOGR., 14, 547-558.

WHITNEY, L.V. (1938). Microstratification of inland lakes. TRANS. WIS. ACAD. SCI. ARTS LETT., 31, 155-173.

WINOGRADSKY, S. (1888). Zur morphologie und physiologie der Schwefelbterien. A. Felix, Leipzig.

WUJEK, D.E., GRETZ, M. & WUJEK, M.G. (1977). Studies on Michigan Chrysophyceae IV. THE MICHIGAN BOTANIST, 16, 191-194.

WUJEK, D.E., HAMILTON, R. & WEE, J. (1975). Studies on Michigan Chrysophyceae III. MICHIGAN BOTANIST, 14, 91-94.

WUJEK, D.E. & VAN DER VEER, J. (1976). Scaled chrysophytes from the Netherlands including a description of a new variety. ACTA BOT. NEERL., 25, 2, 179-190.

Appendix I

CROOME, R.L. and TYLER, P.A. (1983a). *Mallomonas plumosa* (Chrysophyceae), a new species from Australia. BR. PHYCOL. J., 18,151-158.

Appendix II

CROOME, R.L. and TYLER, P.A. (1983b). *Mallomonopsis tasmanica* sp. nov. (Chrysophyceae) and *Mallomonopsis elliptica* Matvienko from Australia. BR. PHYCOL. J., 18, 357-365.

Appendix III

CROOME, R.L. and TYLER, P.A. (1983c). *Mallomonas morrisonensis* (Chrysophyceae), a new species from Australia. BR. PHYCOL. J., 18, 383-389.

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Appendix IV

CROOME, R.L. and TYLER, P.A. (1984a). Microbial micro-stratification and crepuscular photosynthesis in meromictic Tasmanian lakes. V.I.V.L. (in press).

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Appendix V

CROOME, R.L. and TYLER, P.A. (1984b). The micro-anatomy and ecology of *Scourfieldia caeca* (Korsh.) Belcher et Swale in two meromictic lakes in Tasmania. BR. PHYCOL. J. (Submitted).

THE MICROANATOMY AND ECOLOGY OF *SCOURFIELDIA CAECA* (KORSH.) BELCHER ET SWALE IN TWO MEROMICTIC LAKES IN TASMANIA

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ABSTRACT

The minute green flagellate Scourfieldia caeca (Korsh.) Belcher et Swale occurs in large numbers in two dystrophic, meromictic lakes in Tasmania, constantly occupying a narrow stratum immediately above the chemocline, where physical and chemical conditions change dramatically with depth, and where available light is less than 1% of surface radiation and is restricted to red wavelengths. In its ultrastructure it resembles cultured material described previously but differs in the structure and size of its chloroplast, and in the structure of its diagnostic keel of starch. The electron micrographs of our wild material confirm the detailed observations, by light microscopy, of natural populations by earlier workers.

INTRODUCTION

The genus Scourfieldia was established by G.S. West (1912) when he described a minute green flagellate, S. complanata, collected from a pond on Leyton Flats, Essex, by D.J. Scourfield. Three other species of the genus, S. cordiformis Takeda 1916, S. quadrata Pascher 1927 and S. conica Schiller 1954 were subsequently described. Korshikov (1916) described an organism from near Kharkov as Cardiomonas caeca,but Huber-Pestalozzi (1961) recognised it as a species of Scourfieldia, naming it S. magnopyrenoidea, after its conspicuous keel of starch (see below). In accordance with the International Code of Botanical Nomenclature (1955), it was amended to Scourfieldia caeca by Belcher and Swale (1963). A marine species, S. marina Throndsen 1969, was described from the coastal waters of Norway, but this organism was subsequently re-named Pseudoscourfieldia marina (Throndsen) Manton 1975. Thus, the genus as presently construed contains 5 species, S. complanata, S. caeca, S. cordiformis, S. quadrata and S. conica. The genus is allied with the Loxophyceae (e.g. Moestrup, 1982) or Prasinophyceae (e.g. Norris, 1980). Manton (1975) regards the taxonomic position of Scourfieldia as uncertain because there are few microanatomical observations; in any case, the validity of the Loxophyceae and Prasinophyceae are under debate.

The only member of the genus which has been examined electron-microscopically is S. caeca, by Manton (1975) and Melkonian and Preisig (1982), using transmission microscopy only. Ecological information is scant. Scourfield reported to West (1912) that S. complanata "occurred in myriads, the water of the pond being distinctly green without any noticeable alteration in intensity from about the middle of January to the middle of April 1912. This period included one week of severe frost when the pond was covered with fairly thick ice. The green colour of the water was due entirely to this one organism." Later, Scourfield (1944) reported

S. complanata from three bomb craters in Epping Forest while Lund (1942) reported that the same organism was often abundant from January to April, "exclusively above the bottom deposits and not in the plankton", in Leg-Of-Mutton Pond, Richmond Park, Surrey. For S. caeca we have only the observations that the organism occurred in ice-covered water in the hole left by a fallen tree in the English Lake District (Belcher and Swale, 1963), in two temporary ponds in Lancashire (Belcher, 1964) and in a small pond near Cambridge (Melkonian and Preisig, 1982).

S. caeca occurs in two meromictic lakes in the South-west wilderness area of Tasmania. The two lakes, Lake Fidler and Sulphide Pool, both have an intense physico-chemical stratification, with a finely structured microbial array across the chemocline. The general features of the lakes and their microorganisms have been described elsewhere (King and Tyler, 1981, 1982, 1983; Croome and Tyler, 1983a,b, 1984). Scourfieldia caeca is a prominent and constant component of the stratified microbial community in both lakes. Here, we describe the microanatomy and eco-physiology of these two wild populations of S. caeca.

MATERIALS AND METHODS

Most methods have been published in previous descriptions of the lakes (e.g. King and Tyler, 1981; Croome and Tyler, 1984). In this study photosynthetically active radiation (PAR) was measured with a Lambda LiCor quantameter, and its spectral distribution was determined with a Techtum QSM-2500 underwater quantaspectrometer. The in vivo absorption spectrum of S. caeca was determined by filtering cells onto cellulose nitrate filters, clearing the filters with cedar oil, and examining them with a Pye-Unicarn SP8-100 spectrophotometer. Specimens for scanning electron microscopy (SEM) were exposed to 1% osmium tetroxide vapour for 20-30 seconds,

placed on polylysine-coated glass coverslips (Marchant and Thomas, 1983), dehydrated in an acetone series, critical point dried using CO_2 as the intermediary fluid, sputter coated with gold, and viewed using a Philips 505 SEM. Specimens for direct observation by transmission electron microscopy (TEM) were fixed in 1% glutaraldehyde, air-dried on Formvar-coated copper grids, shadowed with platinum/palladium (2 parts to 1), and viewed using an Hitachi H-300 TEM. Material for ultrathin sectioning was concentrated by centrifugation, fixed in 1% glutaraldehyde and 1% osmium tetroxide, dehydrated in acetone, and embedded in Spurr's resin. After sectioning the material was placed on Formvar-coated copper grids, stained with 1% uranyl acetate and Reynold's lead citrate, and viewed using an Hitachi H-300 TEM.

OBSERVATIONS

Light microscopy

By light microscopy there is no perceptible difference between cells from the two lakes. From their general morphology they are readily identified as Scourfieldia caeca from published descriptions, notably Korshikov (1916) and Belcher and Swale (1963). Cell size and shape vary considerably but in general our cells are somewhat smaller than those previously described. In face view they range from almost square, through ovoid, to almost circular, 3.0-5.0 µm long and 2.5-3.8 µm wide. They are compressed, appearing cylindrical to ovoid in side view, around 1 µm wide. Flagella are long and subequal, the longer one 16-25 µm in length, the shorter 10-23 µm. The difference in length between the two is usually 5-6 µm. Several stages of division have been observed, including cells with four complete flagella but no sign of cleavage. The crescentic keel, presumably of starch, is large, and conspicuous. The large nucleus and several small granules are

also visible. The cells swim backwards, with flagella trailing. When seen in face view the leading (posterior) end of the cell wobbles rapidly from side to side, giving the cell a box-shaped appearance. The trailing flagella appear practically motionless because they are beating in the vertical plane (Belcher, 1964). Our observations do not accord with those of Melkonian and Preisig (1982) who state that the cell body rotates during swimming.

Electron Microscopy

Scanning and transmission electron micrographs of Tasmanian specimens are shown in Figs. 1-6. In most features our observations on wild cells agree with those of Manton (1975) for cultured cells. The main differences are (1) a pattern of shallow grooves occurs in the cell membrane, visible in both scanning (Fig. 1) and transmission (Fig. 3) micrographs, (2) the chloroplasts of our material occupy a much larger proportion of the cell, (3) prominent osmiophilic globules, probably lipid, occur in the cytoplasm (Fig. 3), and (4) the keel, presumably starch, is practically homogeneous in composition, not made up of "relatively dense masses of semi-opaque material interspersed with granules of unknown nature" (Manton, 1975). The appearance of the keel in cells out suggests that it is a single, large starch grain (Fig. 3).

Ecology - Lake Fidler

In common with all meromictic lakes, Lake Fidler displays large changes in physical and chemical conditions across the chemocline. Full details, including seasonal variation, are given by King and Tyler (1982). A typical example is shown here in Figure 7. The sharpest manifestation of the chemocline is the abrupt change in redox potential. On 6/4/82 this occurred at 3.1 m below the surface, though its depth is dependent on lake

water level and other factors (Bowling and Tyler, 1984). Irrespective of the actual depth of the redoxcline, in the zone immediately above it oxygen concentrations fall to zero and pH and salinity increase considerably. (Fig. 7 and King and Tyler, 1982). Below it, sulphide concentrations increase dramatically. Across this interface there is an array of microorganisms, each species occupying a comparatively narrow stratum. The principal components of this community are the flagellates Trachelomonas volvocina Ehrenb. and Scourfieldia caeca, the colourless sulphur bacteria cf. Achromatium and Beggiatoa, the bacterial consortium "Chlorochromatium aggregatum" Lauterborn, and the green photosynthetic sulphur bacterium Chlorobium (Croome and Tyler, 1984). Though its population may wax and wane Scourfieldia caeca is always present, characteristically positioned by both day and night in a narrow stratum (≈0.05 m thick) peaking 0.05-0.30 m above the redox shift (Fig. 8). In this position physical and chemical conditions are changing rapidly with depth (Fig. 7 and King and Tyler, 1982), but not with season. The ranges of values at the depth of the population peak during this study (January 1981-April 1983) were pH 6.1-7.1, K_{18} 435-1670 μmho cm^{-1}, dissolved oxygen 0-3.0 mgL^{-1}, temperature 7.9-17.8°C. The maximum cell concentrations of S. caeca ranged from $3.5x10^3$ to $860x10^3$ cells per ml, contributing to Chlorophyll a values as high as 850 μgL^{-1} at these depths (Fig. 7). Although S. caeca is always present in this zone its population density can vary from place to place in the lake by an order of magnitude.

Ecology - Sulphide Pool

In the more sheltered, pond-like Sulphide Pool the same general microbial zonation occurs across the chemocline with the principal exception that no colourless sulphur bacteria are present. Scourfieldia caeca again occupies a position immediately above the redoxcline but the

population is usually dispersed through a greater depth. Occasionally, however, it is concentrated in a precise, narrow zone (Fig. 8). During this study, cell densities at the population peak ranged from <200-1.3x10^6 cells ml^{-1}, with maximum Chlorophyll *a* values in excess of 1200 µgL^{-1}. The range of pH at the population maximum was 5.4-5.7, K_{18} 113-730 µmho cm^{-1}, dissolved oxygen <0.1-1.8 mgL^{-1}, and temperature 6.8-15.3°C.

Ecology - Light Climate

Both lakes are highly dystrophic so that downwelling photosynthetically-active radiation (PAR) is rapidly attenuated and limited to the red end of the spectrum (King and Tyler, 1982, 1983; Croome and Tyler, 1984). At the depths of maximum populations of *S. caeca* in both lakes the amount of PAR present is always less than 1% of surface values and restricted to wavelengths above 550 nm. Underwater quantaspectroradiometry on 2/2/81 (Fig. 9), when the population of *S. caeca* peaked at 570x10^3 cells ml^{-1} between 1.40 and 1.45 m, showed a significant transmission trough at about 650-690 nm at depths below 1.3 m. An *in vivo* spectrophotometric scan of *S. caeca* showed (Fig. 9) that this trough resulted from absorption of PAR at that wavelength by the Chlorophyll *a* of *S. caeca*. Figure 9 also shows that in our lakes the flagellate must rely for photosynthesis almost entirely on light energy harvested at this wavelength.

DISCUSSION

In the initial investigations of Lake Fidler and Sulphide Pool, King and Tyler (1982, 1983) noted that the upper region of the chemocline was occupied by "large numbers of a minute flagellate of bacterial proportions, with long, apparently isokont flagella, probably of chlorophyte affinities." This study has shown that the organism is the chlorophyte *Scourfieldia caeca*.

It is present in the lakes in such large numbers as to permit for the first time electron microscopy of a wild population of the flagellate, with fixation being carried out within minutes of sampling. The ultrastructure of our wild material is similar in most respects to that of cultured cells described by Manton (1975). One difference is that in our cells the chloroplast occupies a much larger proportion of the cell and has many more thylakoids. Such a finding is not unexpected as Vesk and Jeffrey (1977) have shown that chloroplast structure in algal cells varies, depending on the nature of the lighting. One would expect that cells growing in crepuscular conditions ~~(Croome and Tyler, 1984)~~ would have larger chloroplasts than cells grown under the well-lit conditions of culture.

The second significant difference between Manton's cells and those pictured here concerns the structure of the "keel" present in the chloroplast. Earlier workers referred to a large keel of starch. After examining culture material, Manton emended the description of the keel, showing that starch was present only at its centre, most of the keel being made up of "relatively dense masses of semiopaque material interspersed with granules of unknown nature." The observations reported here, of naturally occurring cells, contradict the findings of Manton, and agree with the light microscope observations of earlier works. The keel in cells of S. caeca from Lake Fidler and Sulphide Pool is almost homogeneous and its appearance is consistent with the hypothesis that it is a single large starch grain.

Manton (1975) made no mention of osmiophilic globules in her cells. They are very obvious by light and electron microscopy in our material and it was probably similar granules which Korshikov figured in his description of a Russian population. Manton did not observe grooves in the cell membrane though some of her micrographs (e.g. Figs. 19,22) contain a hint of such structure.

An unusual aspect of the ecology of Scourfieldia caeca in our lakes is its selection of such dimly lit strata, with PAR levels much less than the 1% level, traditionally regarded as the light level at which photosynthesis just balances respiration. At these depths, the organism is further restricted in that the available light is all at wavelengths greater than 550 nm. The constant presence of this active organism, both by day and by night, in a characteristic position just above the chemocline suggests that there is distinct advantage in being there. We suspect that the benefit is the availability of nitrogen and or phosphorus. No nutrient analyses were carried out during this study but the data of King and Tyler (1982) suggest a considerable increase of phosphorus across the chemocline. We presume that the cells are adapted to the low light levels and restricted spectrum. Preliminary investigations with in situ incubation with $NaHC^{14}O_3$ show that considerable incorporation of inorganic carbon takes place, but we cannot rule out the possibility of supplementary organotrophic nutrition.

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References

BELCHER, J.H., 1964. Further notes on Scourfieldia caeca. Br. Phycol. Bull., 2: 371-373.

BELCHER, J.H. & SWALE, E.M.F., 1963. Some new and uncommon British Volvocales II. Br. Phycol. Bull., 2: 210-218.

BOWLING, L.C. & TYLER, P.A., 1984. Endangered lakes of scientific and cultural value in the world heritage area of south-west Tasmania. Biol. Cons. (in press).

CROOME, R.L. & TYLER, P.A., 1983a. Mallomonopsis tasmanica sp. nov. (Chrysophyceae) and Mallomonopsis elliptica Matvienko from Australia. Br. phycol. J. (in press).

CROOME, R.L. & TYLER, P.A., 1983b. Mallomonas morrisonensis (Chrysophyceae) a new species from Australia. Br. phycol. J. (in press).

CROOME, R.L. & TYLER, P.A., 1984. Microbial microstratification and crepuscular photosynthesis in meromictic Tasmanian lakes. Verh. Internat. Verein. Limnol. (in press).

HUBER-PESTALOZZI, G., 1961. Das Phytoplankton des Susswassers. Die Binnengewasser, 16, 5.

KING, R.D. & TYLER, P.A., 1981. Meromictic lakes of south-west Tasmania. Aust. J. Mar. Freshwater Res., 32: 741-756.

KING, R.D. & TYLER, P.A., 1982. Lake Fidler, a meromictic lake in Tasmania. Arch. Hydrobiol., 93: 393-422.

KING, R.D. & TYLER, P.A., 1983. Sulphide Pool and Lake Morrison, meromictic lakes of south-west Tasmania. Arch. Hydrobiol., 96: 139-163.

KORSHIKOV, A.A., 1916. Materialy k flore vodorostei Rossii. Cardiomonas caeca nov. gen. et sp. iz gruppy zelenykh Flagellata. J. Microbiol., Petrograd, 3: 61-68: 233-234.

LUND, J.W.G., 1942. Contributions to our knowledge of British algae VIII. J. Bot., Lond., 80: 57-73.

MANTON, I., 1975. Observations of the microanatomy of Scourfieldia marina Throndsen and Scourfieldia caeca (Korsh.) Belcher et Swale. Arch. Protistenk., 117: 358-368.

MARCHANT, H.J. & THOMAS, D.P., 1983. Polylysine as an adhesive for the attachment of nanoplankton to substrates for electron microscopy. J. Micros., 131: 127-129.

MELKONIAN, M. & PREISIG, H.R., 1982. Twist of central pair microtubules in the flagellum of the green flagellate Scourfieldia caeca. Cell Biol. Int. Repts., 6: 269-277.

MOESTRUP, O., 1982. Flagella structure in algae: a review, with new observations particularly on the Chrysophyceae, Phaeophyceae (Fucophyceae), Euglenophyceae, and Reckertia. Phycologia, 21: 4: 427-528.

NORRIS, R.E., 1980. Prasinophytes. In Developments in Marine Biology. Vol. 2. Phytoflagellates (Cox, E.R., editor), 85-145. Elsevier, New York.

SCOURFIELD, D.J., 1944. The nannoplankton of bomb-crater pools in Epping Forest. Essex Nat., 27: 231-241.

VESK, M. & JEFFREY, S.W., 1977. Effect of blue-green light on photosynthetic pigments and chloroplast structure in unicellular marine algae from six classes. J. Phycol., 13: 280-288.

WEST, G.S., 1912. Algological notes X-XIII. J. Bot., Lond., 50: 321-331.

Figures

Figs. 1-3. Electron microscopical observations of *Scourfieldia caeca* from Sulphide Pool.

Fig. 1. SEM of whole cell. Flagella were lost during fixation. g=grooves in cell membrane. Scale bar denotes 1 μm.

Fig. 2. TEM of whole cell. Note long subequal flagella with hair-point extensions. Scale bar denotes 5 μm.

Fig. 3. Longitudinal section of cell in face view. a = apical notch, c = chloroplast, g = groove in cell membrane, k = keel of "starch" in chloroplast, o = osmiophilic globules, n = nucleus. Scale bar denotes 1 μm.

Figs. 4-6. Ultrathin sections of *Scourfieldia caeca*. Scale bars denote 1 μm.

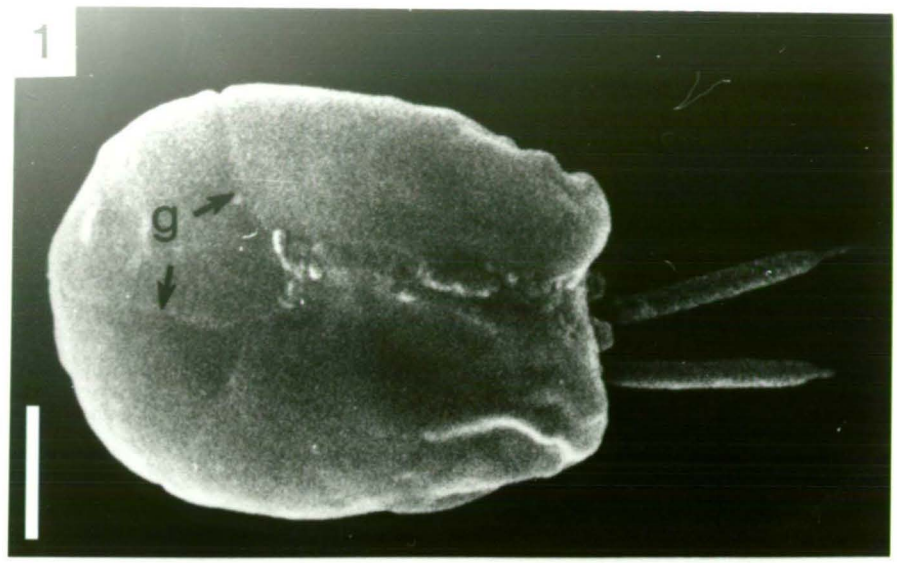
Fig. 4. Longitudinal section in side view. a = apical notch, k = keel of "starch", n = nucleus.

Figs. 5 & 6. Transverse sections of anterior end of cell, showing the relationship between the cell, its apical groove, and insertion of the flagella. a = apical groove, c = chloroplast, f = flagella, fb = flagella bases, n = nucleus.

Fig. 7. Physicochemical and microbial profiles across the chemocline in Lake Fidler, 6 April 1982. The broken line indicates the position of the redoxcline. DO_2 = dissolved oxygen, Eh = redox potential, $S^{=}$ = dissolved sulphide, Chl a = chlorophyll *a*, BChl d = Bacteriochlorophyll *d*.

Fig. 8. The spectrum of PAR at selected depths in Sulphide Pool, 2 Feb. 1981. The broken line indicates the *in vivo* absorption spectrum of the population of *S. caeca*.

Fig. 9. Selected distribution profiles of *S. caeca* from Lake Fidler and Sulphide Pool. The broken line indicates position of the redoxcline.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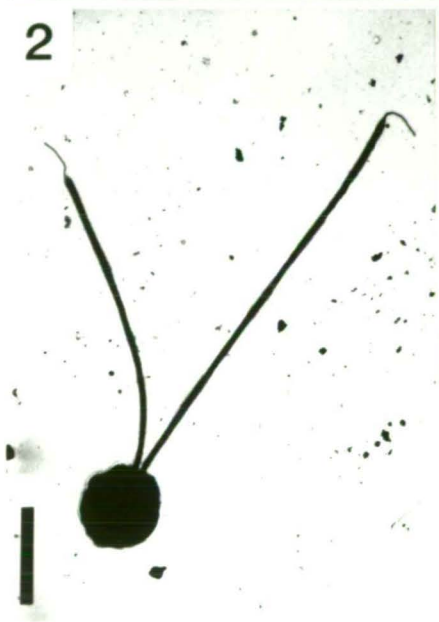
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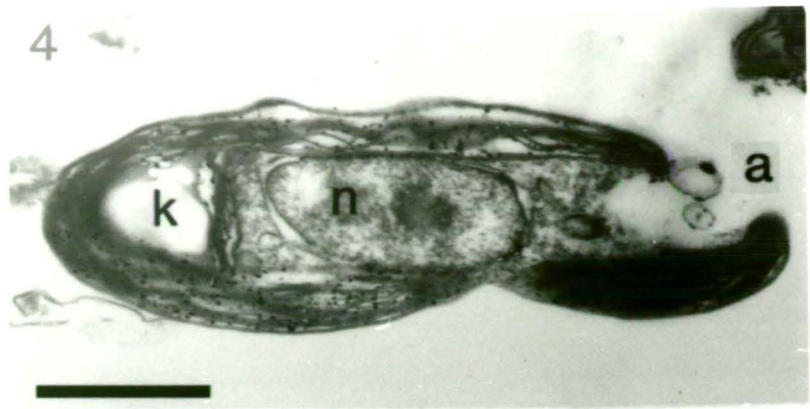

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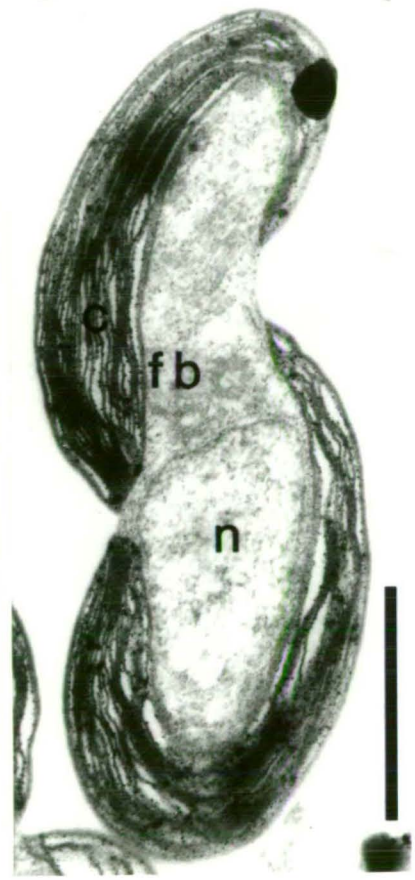

Appendix VI

CROOME, R.L. and TYLER, P.A. (1984c). The micro-anatomy and ecology of "*Chlorochromatium aggregatum*" in two meromictic lakes in Tasmania. JOURNAL OF GENERAL MICROBIOLOGY (Submitted).

The Micro-anatomy and Ecology of "Chlorochromatium aggregatum" Lauterborn in two Meromictic Lakes in Tasmania

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Australia.

Running Title: "Chlorochromatium aggregatum" in Tasmanian lakes.

Subject Category: Ecology

SUMMARY

The motile ectosymbiotic consortium "Chlorochromatium aggregatum" occurs in large numbers in two meromictic lakes in Tasmania. In its structure it resembles specimens previously described, except that vesicles or mesosomes are present in the central bacterium. The population occupies a narrow stratum near the chemocline, but may occur either above or below it, in microaerobic or anaerobic conditions respectively. Its presence under aerobic conditions need not invalidate previous hypotheses concerning the mutualistic relationship between the two partners of the consortium.

INTRODUCTION

Several bacterial consortia have been described in which a central, chemoorganotrophic, motile bacterium is covered by synchronously dividing sulphur bacteria (Trüper and Pfennig, 1978). The Chlorobiaceae are the only bacteria known to form such ectosymbiotic associations. The consortia were originally described as species. However, this is "without standing in nomenclature" (Truper and Pfennig, 1971). The names are still useful however, and are freely used in the literature; where they appear here they are placed in inverted commas.

The best example of such consortia is "Chlorochromatium aggregatum" Lauterborn, in which cells of green sulphur bacteria are regularly arranged around a large, colourless, polarly flagellated, rod-shaped bacterium of unknown physiological activity and taxonomic position (Stanier et al., 1981). There is little doubt that the green bacteria belong to the genus Chlorobium (Trüper and Pfennig, 1971). Growth and division of all cells occur synchronously, indicating a high degree of metabolic interdependence.

Recently, Stanier et al. (1971) and Gorlenko et al. (1977) have published light micrographs of "C. aggregatum" and Caldwell and Tiedje (1975) have published electron micrographs of ultrathin sections. Pfennig (1980) has discussed it in a review article on syntrophic mixed cultures and symbiotic consortia. In the latter work, the hypothesis is presented that the central bacterium is either a sulphate- or sulphur-reducing bacterium. However, repeated attempts at culturing the central bacterium in isolation have failed. One possible reason for this is that the central bacterium of the consortium functions as an electron donor,

and is obligately dependent on the green sulphur bacteria as electron acceptors: growth of the central bacterium in pure culture would therefore be impossible (Pfennig, 1980).

"Chlorochromatium aggregatum" and other, less known consortia (see Starr and Schmidt, 1981) exhibit chemo- and photo-tactic responses and accumulate in conditions of optimum sulphide concentrations and light intensity (Pfennig, 1978). They have been found only in freshwater habitats, and may occur both in shallow pools above the mud and in the layers of green or purple sulphur bacteria in stratified lakes.

"Chlorochromatium aggregatum" occurs in two dystrophic, meromictic lakes in the south-west wilderness area of Tasmania, Australia. The two lakes, Lake Fidler and Sulphide Pool, both have severe physico-chemical stratification at a depth of 2-3 m, with a finely-structured microbial array across this chemocline. The general features of the lakes and their microorganisms have been described elsewhere (King and Tyler, 1981, 82,83; Croome and Tyler, 1983a,b, 1984a,b). "Chlorochromatium aggregatum" is a prominent member of the stratified microbial community in both lakes, occurring at dimly-lit depths in the vicinity of the chemocline. Here, we describe its micro-anatomy and ecophysiology in these meromictic lakes.

METHODS

Most field and analytical methods have been published in previous descriptions of the lakes (e.g. King and Tyler, 1981; Croome and Tyler, 1984a). The physico-chemical zonation and accompanying microbial array was sampled with a close-interval pneumatic sampler (Croome and Tyler, 1984a). Specimens for scanning electron microscopy (SEM) were exposed to 1% osmium tetroxide vapour for 20-30 seconds, placed on polylysine-coated glass coverslips (Marchant and Thomas, 1983), dehydrated in an acetone series, critical point dried using CO_2 as the intermediary fluid, sputter coated with gold, and viewed using a Philips 505 SEM. Material for ultra-thin sectioning was concentrated by centriguation, fixed in 1% glutaraldehyde and 1% osmium tetroxide, dehydrated in acetone, and embedded in Spurr's resin. After sectioning the material was placed on Formvar-coated copper grids, stained with 1% uranyl acetate and Reynold's lead citrate, and viewed using an Hitachi H-300 TEM.

RESULTS

Light micrographs and scanning electron micrographs of the consortium are shown in Figs. 1-5. By light microscopy there is no perceptible difference between consortia from the two lakes. The consortium appears as a slightly knobbly, square to rectangular shape (Figs. 1-2), grey under phase contrast microscopy, which slowly revolves about its long axis as it swims along an undulating path. Recently divided consortia are approximately cubical, about 2 μm long by 1.5 μm wide. Dividing consortia are more elongate. Scanning EM shows that in non-dividing consortia the outer, photosynthetic bacteria are arranged more or less at right angles to the long axis (Fig. 3) though in elongate, dividing consortia some cells may be randomly orientated (Fig. 5) or almost longitudinally arranged (Fig. 4). Although the consortia are motile, we have never seen flagella by either light or electron microscopy.

By TEM (Figs. 6-10) the internal structure of the green photosynthetic bacteria of the outer layer is similar to that of the free-living Chlorobium populations in the lakes (see below). The cells are of the Chlorobium limicola type and the characteristic chlorobium vesicles are quite apparent.

The central bacterium is comparatively long, occasionally exceeding 3 μm in length, and of the order of 0.5-1.0 μm in diameter. The cell wall is often in close contact with those of the photosynthetic bacterial cells around it, and displays structures similar to those described by Caldwell and Tiedje (1975) as hexagonal cups, postulated to play a role in maintaining the integrity of the consortium (Figs. 6-7). The matrix of the central bacterium is lacking in detail, but large vesicles or mesosomes were observed near the cell wall (Figs. 8-9). Such structures have not been reported previously for the central bacterium, and their function is unknown.

Lake Fidler and Sulphide Pool display abrupt changes in physical and chemical conditions across the chemocline, typical of meromictic lakes. Seasonal variation in these conditions has been described by King and Tyler (1982, 83). Figure 11 shows a typical example, from Lake Fidler, of this physicochemical zonation and of the accompanying microbial array. The sharpness of the chemocline is best demonstrated by the almost horizontal redoxcline, above which there is usually a microaerobic zone. The microbial array consists of the flagellates Trachelomonas volvocina Ehrenb. and Scourfieldia caeca (Korsh.) Belcher et Swale, the colourless sulphur bacteria cf. Achromatium and Beggiatoa, the free-living

photosynthetic bacterium Chlorobium, and "Chlorochromatium aggregatum". All of these except "Chlorochromatium" maintain a more or less constant position, relative to the chemocline, diurnally and throughout the year. "Chlorochromatium", on the other hand, varies its position from high in the microaerobic zone (0.25 m above the redoxcline), at an oxygen concentration of 0.4 mg/L and a redox potential of +350 mV, to well within the anaerobic zone, with a sulphide concentration of 30 mg/L and a redox of -100 mV. Between January 1981 and April 1983 numbers ranged from 100 - 64×10^4 consortia per mL at the depth of the population peak, usually occupying a layer about 0.1 m thick.

Though the position of the chemocline relative to lake surface varies with water level it is always at such a depth that light is severely attenuated and restricted to wavelengths >550 nm by the dystrophic water (King and Tyler, 1982; Croome and Tyler, 1984b). The population of "Chlorochromatium aggregatum" always receives much less than 1% of surface photosynthetically-active radiation.

"C. aggregatum" also occurs in Sulphide Pool but it was not observed there before April 1982. It occurs there under similar environmental conditions and occupies the same niche as in Lake Fidler. It is possible that it was introduced to Sulphide Pool by sampling equipment previously used in Lake Fidler.

DISCUSSION

The structure of "C. aggregatum" from Lake Fidler is similar to that found by previous workers, with cells of Chlorobium enveloping and being in close contact with a large central bacterium. It is also similar in its ultrastructure, the photosynthetic symbionts possessing distinct chlorobium vesicles, and the central bacterium being surrounded by structures which may help to keep the consortium together (Caldwell and Tiedje, 1975). The vesicles or mesosomes observed in the central bacterium have not been reported previously, and their function is unknown.

The consortium "Chlorochromatium aggregatum" has been found in large numbers in several other stratified lakes. In Lake Sakavo (Gorlenko and Chebotarev, 1981) and Lesnaya Lamba (Dubinina and Kuznetsov, 1976) it occurred at the same depth as free-living green photosynthetic bacteria, in numbers up to 420,000 and 100,000 consortia/mL respectively. In Lake Nesoytjern it was recorded at 30,000 consortia/mL (Blakar, 1979), and in Burke Lake it comprised 10 per cent of the photosynthetic bacterial community (Caldwell and Tiedje, 1975). It has also been reported in

Lakes Mary and Rose (Parkin and Brock, 1980) and in Estonian lakes (Gorlenko and Lokk, 1979). In all cases, it has been reported as inhabiting strictly anaerobic regions.

In Lake Fidler, where concentrations of "C. aggregatum" up to 640,000 consortia/mL have been recorded, the largest population maximum yet reported for this "organism", it has been found under both aerobic and anaerobic conditions. This is interesting in the light of the recent hypothesis (Pfennig, 1980) as to the relationship between the two members of the consortium, that the central organism is a sulphate- or sulphur-reducing bacterium utilizing extracellular sulphur produced by the photosynthetic bacteria, which in turn use dissolved sulphide, produced by the central bacterium, as an electron donor for their anoxygenic photosynthesis. At the same time the Chlorobium cells gain motility, enabling positive phototactic responses. The presence of "C. aggregatum" in the microaerobic zone of Lake Fidler in no way negates Pfennig's hypothesis, but enhances it. When present in the anoxic zone the metabolic activity of the consortium could be just as supposed above. Its presence in the microaerobic zone most likely results from a positive phototactic response, and although the reducing conditions required for anaerobic photosynthesis would not be present outside the consortium, they could well prevail between the cells within it. Why "C. aggregatum" should be found high in the microaerophilic zone in one month, and well into the sulphide zone in another, is not known. Its location is presumably a response to light, redox potential, nutrients and sulphur distribution.

ACKNOWLEDGEMENTS

We thank the Australian Research Grants Scheme and the University of Tasmania for research grants. We thank sincerely Dr. H.J. Marchant and Mr. R. Cruikshank for assistance with specimen preparation, and Dr. D.P. Thomas and Mr. A. Eastgate for assistance during electron microscopy.

REFERENCES

BLAKAR, I.A., 1979. A close-interval water sampler with minimal disturbance properties. Limnology and Oceanography, 24: 983-988.

CALDWELL, D.E. & TIEDJE, J.M., 1975. The structure of anaerobic bacterial communities in the hypolimnion of several Michigan lakes. Canadian Journal of Microbiology, 21: 377-385.

CROOME, R.L. & TYLER, P.A., 1983a. Mallomonopsis tasmanica sp. nov. (Chrysophyceae) and Mallomonopsis elliptica Matvienko from Australia. British phycological Journal, 18: 357-365.

CROOME, R.L. & TYLER, P.A., 1983b. Mallomonas morrisonensis (Chrysophyceae) a new species from Australia. British phycological Journal, 18: 383-389.

CROOME, R.L. & TYLER, P.A., 1984a. Microbial microstratification and crepuscular photosynthesis in meromictic Tasmanian lakes. Verhandlungen Internationale Vereinigung Limnologie, in press.

CROOME, R.L. & TYLER, P.A., 1984b. The microanatomy and ecology of Scourfieldia caeca (Korsh.) Belcher et Swale in two meromictic lakes in Tasmania. British phycological Journal, in press.

DUBININA, G.A. & KUZNETSOV, S.I., 1976. The ecological and morphological characteristics of microorganisms in Lesnaya Lamba (Karelia). Internationale Revue der gesamten Hydrobiologie, 61: 1-19.

GORLENKO, V.M. & CHETABOREV, E.N., 1981. Microbiological processes in the meromictic Lake Sakovo. Microbiology, 50: 98-102.

GORLENKO, V.M. & LOKK, S.I., 1979. Vertical distribution and special composition of microorganisms from some stratified Estonian lakes. Mikrobiologiya, 48: 351-359.

GORLENKO, V.M., DUBININA, G.A. & KUZNETSOV, C.I., 1977. The ecology of aquatic microorganisms. Moscow. Science Publn. Acad. Sciences. U.S.S.R.

KING, R.D. & TYLER, P.A., 1981. Meromictic lakes of south-west Tasmania. Australian Journal of Marine and Freshwater Research, 32: 741-756.

KING, R.D. & TYLER, P.A., 1982. Lake Fidler, a meromictic lake in Tasmania. Archiv fur Hydrobiologie, 93: 393-422.

KING, R.D. & TYLER, P.A., 1983. Sulphide Pool and Lake Morrison, meromictic lakes of south-west Tasmania. Archiv fur Hydrobiologie, 96: 139-163.

MARCHANT, H.J. & THOMAS, D.P., 1983. Polylysine as an adhesive for the attachment of nanoplankton to substrates for electron microscopy. Journal of Microscopy, 131: 127-129.

PARKIN, T.B. & BROCK, T.D., 1980. Photosynthetic bacterial production in lakes: the effects of light intensity. Limnology and Oceanography, 25: 711-718.

PFENNIG, N., 1978. General physiology and ecology of photosynthetic bacteria. In The Photosynthetic Bacteria (Clayton, R.K. & Sistrom, W.R., editors), 3-18. New York. Plenum Press.

PFENNIG, N., 1980. Syntrophic mixed cultures and symbiotic consortia with phototrophic bacteria: a review. In Anaerobes and anaerobic infections (Gottschalk et al., editors), 127-131. Stuttgart. New York. Gustav Fischer Verlag.

STANIER, R.Y., DOUDOROFF, M. & ADELBERG, E.A., 1971. General Microbiology. 3rd Edition. London. Macmillan.

STANIER, R.Y., PFENNIG, N. & TRUPER, H.G., 1981. Introduction to the phototrophic prokaryotes. In The Prokaryotes, Vol. 1. (Starr, M.P. et al., editors), 197-211. Berlin, New York. Springer-Verlag.

STARR, M.P. & SCHMIDT, J.M., 1981. Prokaryote diversity. In The Prokaryotes, Vol. 1. (Starr, M.P. et al., editors), 3-42. Berlin, New York. Springer-Verlag.

TRUPER, H.G. & PFENNIG, N., 1971. Family of phototrophic green sulfur bacteria: Chlorobiaceae Lauterborn; and the taxonomic situation of the consortium-forming species. Request for an opinion. International Journal of Systematic Bacteriology, 21: 8-10.

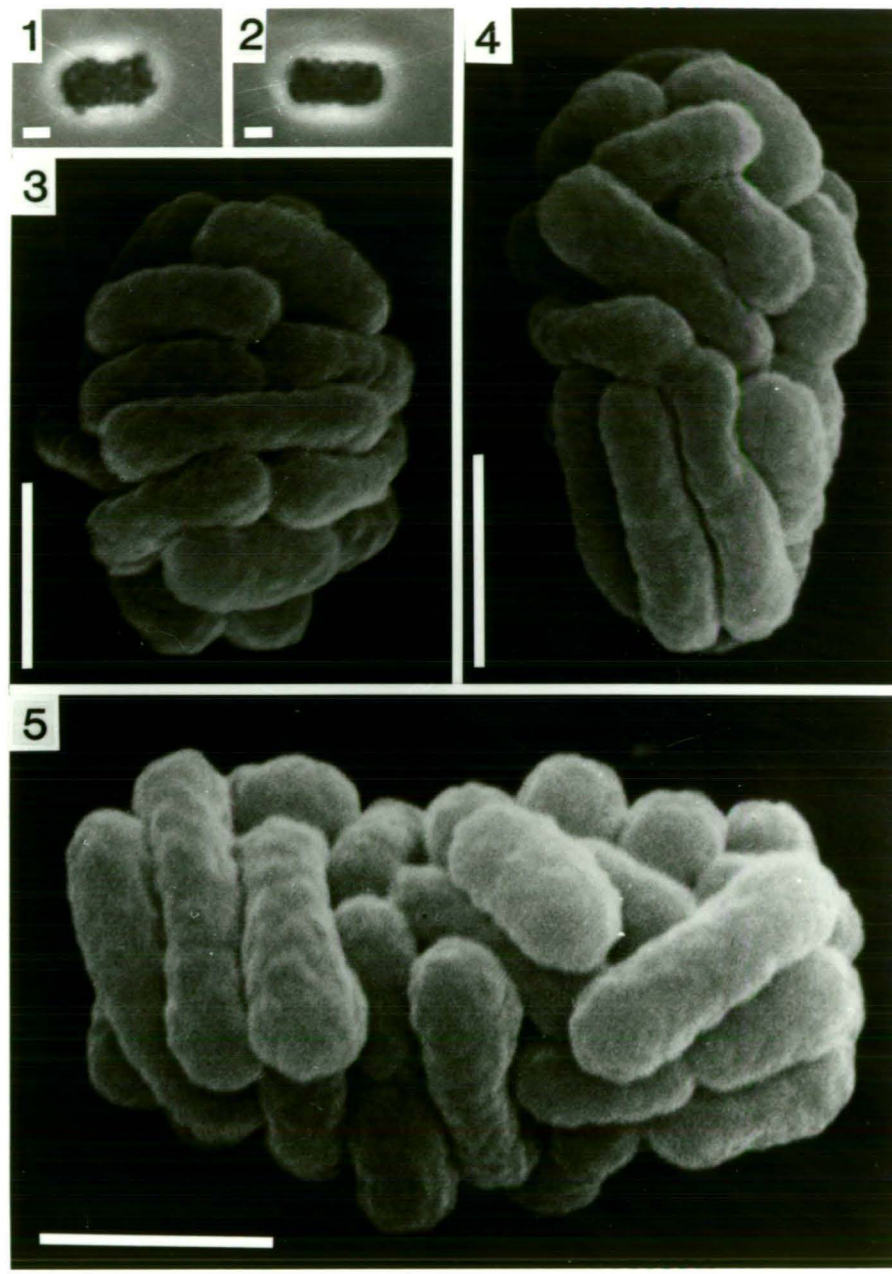
TRUPER, H.G. & PFENNIG, N., 1978. Taxonomy of the Rhodospirillales. In The Photosynthetic Bacteria (Clayton, R.K. & Sistrom, W.R., editors), 19-27. New York. Plenum Press.

Figures

Figs. 1-5. Whole mounts of "Chlorochromatium aggregatum". Figs. 1 & 2. Light micrographs of live, dividing consortia. Figs. 3-5. Scanning electron micrographs of single and dividing consortia. Scale bars denote 1 μm.

Figs. 6-10. Ultrathin sections of "Chlorochromatium aggregatum". Fig. 6. Transverse section of a consortium. Scale bar denotes 1 μm. Fig. 7. Central bacterium enlarged to show surface structures (arrow). Scale bar denotes 0.5 μm. Fig. 8. Oblique section of a consortium. Scale bar denotes 1 μm. Fig. 9. Central bacterium enlarged to show vesicles or mesosomes (arrows). Scale bar denotes 0.5 μm. Fig. 10. Longitudinal section of a dividing consortium. Scale bar denotes 1 μm.

Fig. 11. Physicochemical and microbial profiles across the chemocline in Lake Fidler, 31/1/1981. The broken line indicates the position of the redoxcline. DO_2 = dissolved oxygen, Eh = redox potential, S^{--} = dissolved sulfide, BChl d = bacteriochlorophyll d.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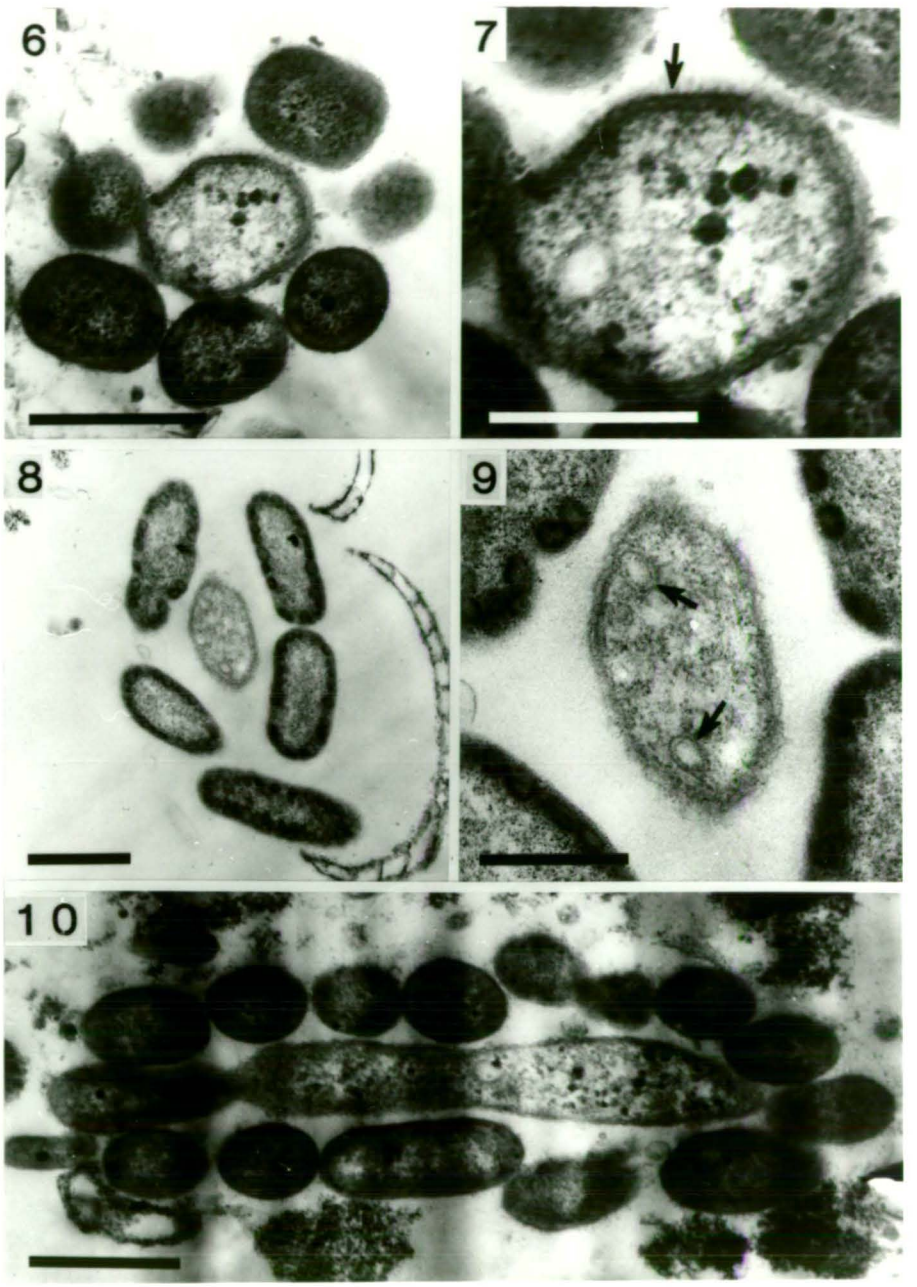
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Appendix VII

CROOME, R.L., DURRSCHMIDT, M. and TYLER, P.A. (1984). A light and electron microscopical investigation of *Mallomonas splendens* (West) Playfair. NOVA HEDWIGIA (Submitted).

A light and electron microscopical investigation of *Mallomonas splendens* (West) Playfair.

by

R. Croome, M. Durrschmidt, and P. Tyler

Key words: Chrysophyceae, Mallomonadaceae, *M. splendens* fa. *splendens*, *M. splendens* fa. *arnhemensis*. Fine structure of scales and bristles.

Abstract: An emended description of Mallomonas splendens (G.S. West) Playfair is given, based on electron microscopical investigations of the cell armour. Two forms - M. splendens fa. splendens and M. splendens fa. arnhemensis are recognized.

Introduction

Mallomonas splendens was first described by G.S. West (1909) as Lagerheimia splendens (Chlorophyceae) from Yan Yean Reservoir, near Melbourne, Australia. Later, Playfair (1912), recognizing its Chrysophyte affinities, transferred it to the genus Mallomonas Perty. Playfair's description was discussed later by Pascher (1925), Conrad (1927) and Krieger (1930), [redacted] and briefly summarized by Huber-Pestalozzi (1941). The descriptions of all these authors agree with each other in most points. M. splendens has been reported only rarely. Besides Australia, where it has been reported from New South Wales (Playfair 1912, 1921, Thomasson 1973) and Victoria (West 1909, Viyakornvilas 1974) it has been found in Holland (Conrad 1933), Java (Huber-Pestalozzi 1941), India (Philipose 1953) and Malaysia (Prowse 1962). All these reports are based on light microscopy.

In the present paper we extend its range to Tasmania and the Northern Territory of Australia and supplement LM observations with scanning and transmission electron microscopy of samples from Australia and Malaya. On the basis of the fine structure of the body scales we describe two formae.

Methods

Plankton was collected with nets (mesh-diameter 5-20 µm) and fixed with formalin or glutaraldehyde. Material for transmission electron microscopy was rinsed with distilled water, one drop placed on formvar-coated grids, air-dried, and then directly studied or shadowed with Platinium/Palladium. For SEM these grids were mounted on aluminium stubs and sputtered with gold, or the specimens were placed on polylysine-coated glass coverslips (Machant & Thomas 1983), dehydrated in an acetone series,

(ie Marchant.)

critical point dried using CO_2 as the intermediary fluid, and sputter-coated with gold. The following microscopes were used: Hitachi H-300 (TEM Figs. ...), Philips 505 (SEM Figs. ...) Department of Botany, University of Tasmania, Hobart/Australia; Philips EM 300 (TEM Figs. ...), Cambridge S4 Stereoscan (SEM Figs. ...) Strahlenzentrum, Justus Liebig University, Giessen, Federal Republic of Germany.

Results

Mallomonas splendens (G.S. West) Playfair emend. Croome, Durrschmidt et Tyler

syn.: Lagerheimia splendens G.S. West (Figs.)

The cells are long and cylindrical. Approximate dimensions are 22-46 µm x 8-13 µm. The cells have a conspicuous cell armour of 3 types of scales. At the anterior end four distinctive scales surround the base of the flimmer flagellum, each scale bearing a long bristle (Fig. ...). For most of its length the cell is covered by spirally-arranged, imbricate body scales. At the posterior end are 4 asymmetric, domed scales, each bearing 1-3 long bristles (Fig. ...). TEM reveals that all scales are ornamented by a closely-spaced sexangular or polygonal mesh, forming a regular alveolar pattern (Fig. ...).

The 4 apical scales (Fig) are oblong or elongate-oval, rounded proximally and slightly attenuated apically. At the anterior end, they have a dome beneath which the foot of the bristle is attached, and, laterally, an area which is less dense than the rest of the scale, and minutely perforated. This area is delimited by a raised, M - shaped ridge (Fig), and admits the shaft of the bristle of the adjacent scale (Fig).

The posterior scales are broadly ovate, with an asymmetrically-placed dome (Fig. ..). SEM micrographs show that the outer surface slopes gently upward from the rest of the scale. In TEM a rib-like structure is seen to delimit the dome against the shield. Figure shows the attachment of bristles beneath the domes of posterior scales. Each scale may bear 1-3 bristles (Fig. ..).

The bristles are of two types. The apical ones are shorter than those of the posterior end (22-35 µm). Both types are open along their length and either taper to a blunt apex, or to a double-toothed apex (Figs. ...). The apical bristles radiate almost at right angles from the cell, while those at the posterior are directed backwards.

The body scales are rhomboidal, with a smooth proximal border and a more-or less-developed V-rib. An anterior submarginal rib is lacking. The alveolar pattern is absent along the outer margin and the scales are ornamented only by irregularly scattered papillae. These papillae are more frequent on the anterior scale surface than on its posterior part (Figs. ...). Cysts were not observed.

Locality: ~~Yan Yean, Victoria~~ Woodford Creek Reservoir, New South Wales; Umbungbung and Kulukuluku Billabongs, Northern Territory; Malaya.

Mallomonas splendens fa. arnhemensis

This fa. splendens
~~The~~ forma differs from ~~the type~~ in that the body scales have a conspicuous annular or ellipsoidal depression in the angle of the V-rib (Figs.). There, the secondary layer is absent and a finely-perforated base plate is visible.

Holotypus: Fig.

The epithet arnhemensis refers to the locality in Arnhem Land, northern Australia.

Locality: Umbungbung and Kulukuluku Billabongs, Northern Territory; Malaya.

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On the basis of light microscopy alone, which does not permit the distinction of the two formae, we report the occurrence of M. splendens in Yan Yean Reservoir in Victoria, Lake Fidler in Tasmania, and in Ross River Reservoir in Queensland.

Discussion

In this paper we emend the description of Mallomonas splendens on the basis of material from Australia and Malaysia. We are unaware of the fate of West's samples from Yan Yean Reservoir, near Melbourne, and the species occurs ther today too sparsely to permit electron microscopy. We have examined Playfair's original samples from waters around Sydney without locating the species. However, a sample from one of Sydney's present-day reservoirs, Woodford Creek, provided abundant material. The scales of these cells (Fig)lack the depression in the angle of the V - rib. We take this to be the taxon which Playfair would have seen, and so adopt it as the fa. splendens.

From the present investigation it is evident that M. splendens, which appears highly distinctive in the light microscope, is also easily distinguished from previously described species on the basis of its ultra-structure. The most distinctive feature is the presence of anterior and posterior whorls of unique scales, to which it is not possible to apply the usual terms shield, flange and dome. As revealed by SEM micrographs, the cone-shaped appearance of the bristle bases in LM is due to the vaulted distal parts of the apical and rear scales, which project somewhat outwards. M. splendens cannot be placed in any of the usual groupings of species of the genus, though in the structure of its body scales it bears some resemblance to M. bronchartiana Compère and to some species of the Quadratae sect. (Momeu & Peterfi 1979).

Two varieties of the species have been described: M. splendens var. pusilla Playfair (syn. M. playfairii Conrad) and M. splendens var. biceps Conrad. Variety pusilla differs from the type in that it has a considerably smaller size and no bristles. As mentioned by Playfair himself, it is possible that it may be found to be only a developmental stage. Conrad's variety biceps is distinguished by radially-directed bristles on both ends, and the prolongation

of the cell apices. In both cases, nothing is known about the fine structure of the cell armour, and on that account there taxonomic position is uncertain.

The two formae of M. splendens described here cannot be distinguished by light microscopy. Electron microscopy reveals that fa. splendens is the only forma in temperate Australia, whereas both formae occur in tropical Australia and Malaysia, sometimes in the same sample.

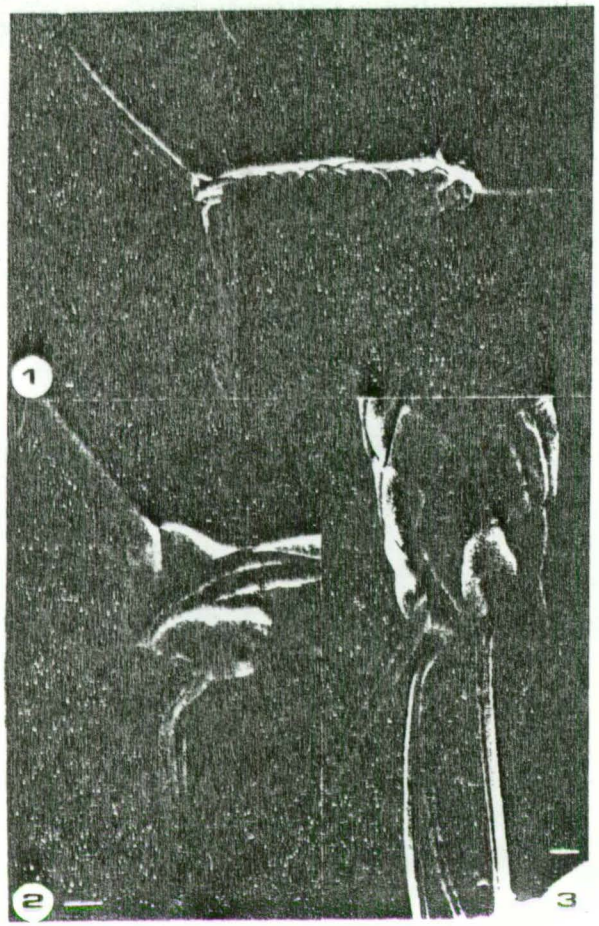
Acknowledgements

We wish to express our sincere thanks to Ms. B. Asmund for her kind help and advice during the preparation of this paper.

One of the authors (M.D.) thanks the 'Strahlenzentrum' Giessen for placing the electron microscopes at her disposal and E. Kaus for translating the latin diagnoses.

We thank the Australian Research Grants Scheme and the University of Tasmania for research grants. Samples from the Northern Territory of Australia were collected while carrying out limnological surveys under contract to the Office of the Supervising Scientist for the Alligator Rivers Region.

We thank Dr. D.P. Thomas and Mr. A. Eastgate for assistance with specimen preparation and operation of electron microscopes, and the National Museum, Sydney, for loan of Playfair's samples. The Sydney Metropolitan Water, Sewerage & Drainage Board kindly provided the sample of Woodford Creek Reservoir and the Melbourne Board of Works those from Yan Yean Reservoir.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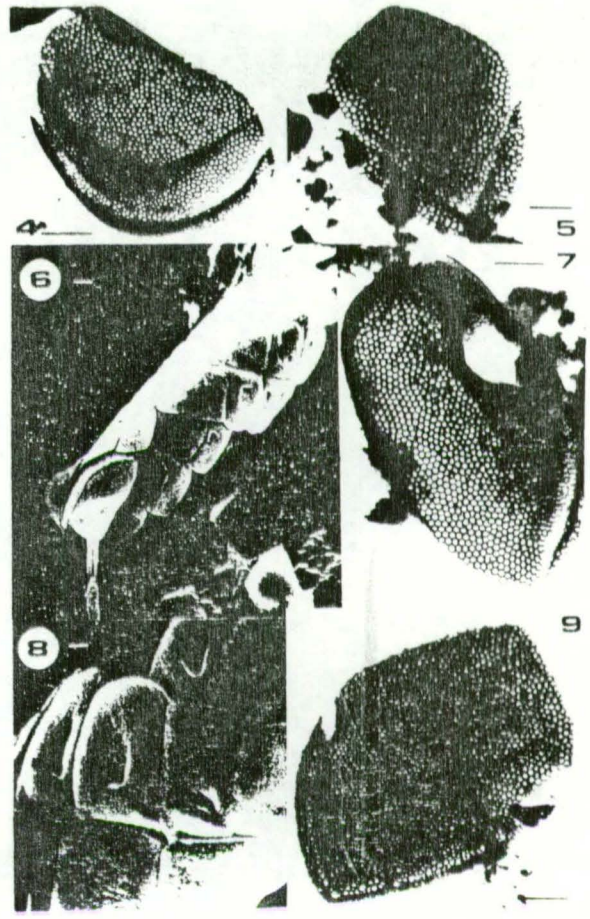
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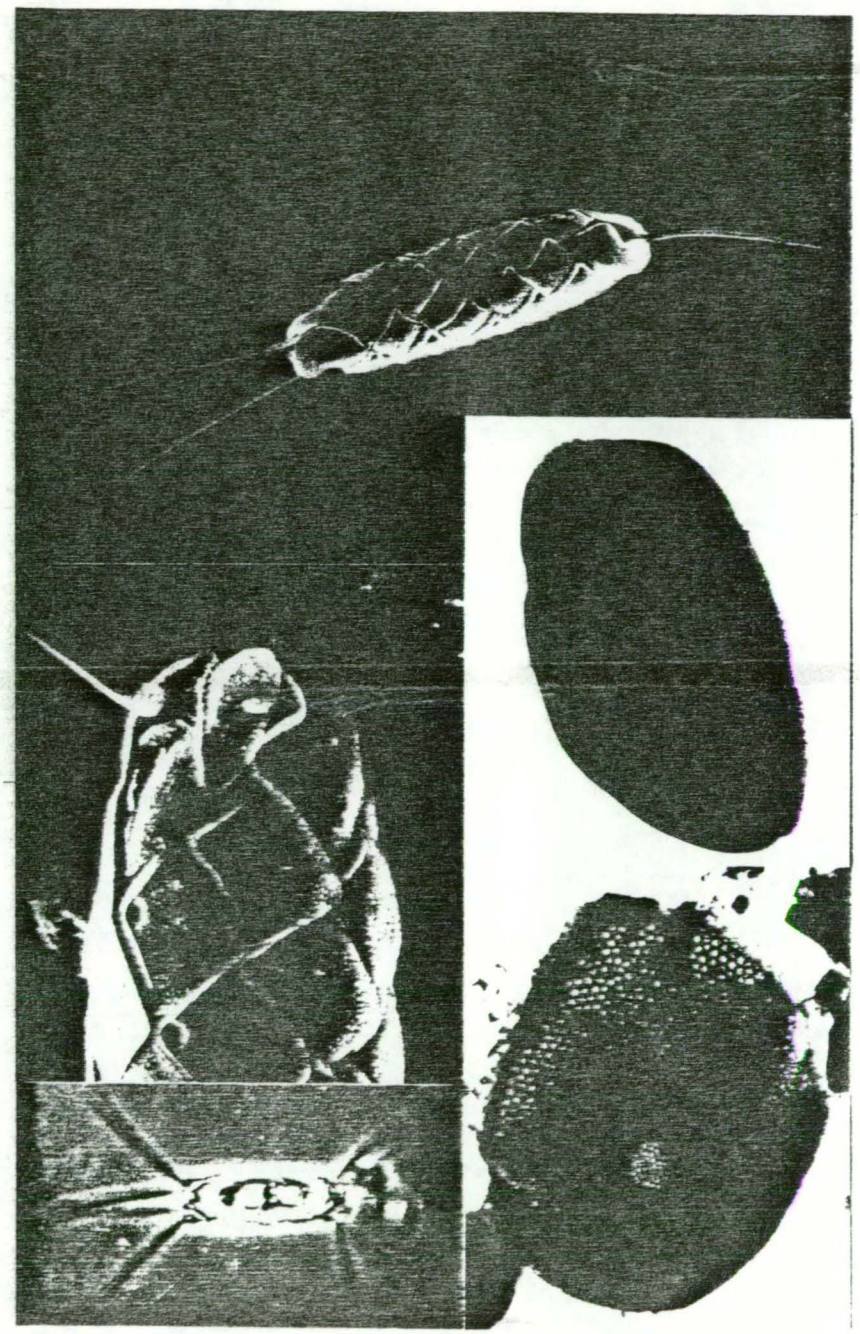


Appendix VIII

DURRSCHMIDT, M. and CROOME, R.L. (1984). Contribution to the knowledge of Mallomonadaceae (Chrysophyceae) from Malaysia and Australia. NORDIC JOURNAL OF BOTANY (Submitted).

Contribution to the knowledge of Mallomonadaceae (Chrysophyceae) from Malaysia and Australia

by

Monika Dürrschmidt & Roger Croome

Abstract

The Mallomonadaceae (Chrysophyceae) of Malaysia and Australia are described by scanning and transmission electron microscopy. Twenty nine taxa are reported from Malaysia, twenty seven for the first time. Seventeen taxa are reported from Australia, twelve for the first time. Twelve species are reported for the first time from the tropics. Four new taxa, Mallomonas ocellata sp. nov., M. tropica sp.nov., M. favosa f. gemina f. nov. and Chrysosphaerella astrea sp.nov. are described, and the diagnosis of Mallomonas adamas Harris & Bradley is emended.

Introduction

Few studies have been made of scale-bearing Chrysophytes from tropical south-east Asia or Australia. There has been only one report from Malaysian waters: Prowse (1962) recorded some twelve taxa of Mallomonadaceae in a light microscope study of freshwater flagellates. In Australia, light microscope observations of the Mallomonadaceae have been made by West (1909), Playfair (1912,1915,1921) and Thomasson (1973), while electron microscope observations have been made by Takahashi (1978), ~~Takahashi & Hayakawa (1979)~~, Croome & Tyler (1983a:b:c), and Ling & Tyler (1984).

The observations reported herein were made during studies designed to investigate, by electron microscopy, the distribution of Mallomonadaceae in Malaysia and Australia. Although preliminary in nature, the investigations have revealed a diverse Chrysophycean flora in the two countries, and several new species have been discovered.

Materials and methods

Plankton was collected with nets (mesh-diameter 5 - 20 um) and fixed with formalin or glutaraldehyde. Material for transmission electron microscopy was rinsed with distilled water, one drop placed on formvar-coated grids, air-dried, and then directly studied or shadowed with Platinium/Palladium. For SEM these grids were mounted on aluminium stubs and sputtered with gold or the specimens were placed on polylysine-coated glass coverslips (Marchant & Thomas 1983), dehydrated in an acetone series, critical point dried using CO_2 as the intermediary fluid, and sputter coated with gold. The following microscopes were Used: Hitachi H-300 (TEM Figs.), Philips 505 (SEM Figs) Department of Botany, University of Tasmania, Hobart/Australia; Philips EM 300 (TEM Figs), Cambridge S4 Stereoscan (SEM Figs) Strahlenzentrum, Justus-Liebig University Giessen, Federal Republic of Germany.

Survey of the species.

Mallomonas adamas Harris & Bradley emend. Durrschmidt & Croome (Figs.).

The cells are spherical to oval in shape, and have three types of scales. The apical scales are depressed obovate, with a conspicuous protuberation which is papillated on one side and smooth on the other (Figs.). The body scales are

distally papillose and proximally smooth (Figs.). The centrally located V-rib noted by Harris & Bradley, in which there is a circular or elongated depression, is in our specimens so asymmetric as to appear as a smooth area across the scale (Figs.). Some of the scales lack the depression in this smooth area (Fig.). In the Malaysian cells the papillated area of the body scales is somewhat vaulted. The posterior scales bear a vertical projection which has a papillated apex (Fig.). In TEM all scales display a hexagonal or polygonal mesh which forms an alveolar pattern (Figs.).

M. adamas has not been reported since its original description from small water bodies in England (Harris & Bradley, 1960).

M. adamas was frequent in Sample 2 from Malaysia, and in Australia occurred in Lake Fidler, Tasmania (April 1982 and April 1983).

Mallomonas annulata (Harris & Bradley) Harris (Figs.)

M. annulata is distributed world-wide and according to Takahashi (1978) is characterized as being stenothermal with oligothermal tendencies. It is reported here from cool temperate waters in Tasmania, Australia, at temperatures around 6°C and tropical waters in Malaysia at around 28°C.

M. annulata was frequent in Malaysian Habitats 1 and 3, and was found in Gumeracha Weir on the River Torrens in South Australia (May 1982) and Lake Leake, Tasmania (August 1971).

Mallomonas aerolata (Figs.)

Missing.

Mallomonas bronchartiana Compère. (Figs.)

This species has been reported from tropical waters only: Lake Chad (Compère, 1975) and Brasil (Cronberg in press).

Scales of M. bronchartiana were seen in Malaysian Habitat 3.

Mallomonas elliptica Matvienko (Figs.)

M. elliptica has been reported world-wide, including tropical habitats (e.g. Compère, 1975).

It is reported here from Kulukuluku and Umbungbung Billabongs, Northern Territory of Australia (Oct. 1980) and from Malaysian Habitats 1 and 2.

The bristles of the Australian specimens are unusual in being very long (up to 60 µm) and having a wide apex with one side prolongated to a triangular tip (see Croome & Tyler, 1983b).

Mallomonas favosa Nicholls (Figs.)

Previously reported only from Canada (Nicholls, 1984). Specimens with typical scales are reported from Malaysian Habitats (Figs.). Three other forms of this taxa were distinguished: the differences of form 1 and 2 are of questionable taxonomic significance but the third form deviates in more than one detail and justifies the erection of a new forma.

Mallomonas favosa form 1 (Figs.)

A conspicuous round pit with a thickened central area is developed in association with the posterior hole of the body scales. A similar pit is often present in the median angle of the submarginal rib. The apical scales have a row of similar pits along the inner edge of the dorsal submarginal rib (Fig. arrow). This ornamentation was seen in both mature and immature cells (Fig.).

This form was observed in Malaysian Habitat 2 only.

Mallomonas favosa form 2 (Figs.)

Several holes are present along the inner edge of the posterior submarginal rib. In addition two rows of papillae adorn the anterior submarginal rib and the anterior flange.

This form was frequent in Malaysian Habitat 2, while scales only were observed in Kulukuluku Billabong, Northern Territory of Australia (Oct. 1980).

Mallomonas favosa f. gemina f.nov.

This forma differs from the type in that the dense system of polygonal apertures on the scales is not visible in SEM (Fig.). The scale surface appears smooth, being ornamented only by small regularly spaced papillae. The scale reticulation is more dense, and the pits are smaller and more numerous and form a row along the submarginal rib.

The anterior flange is smaller and together with the anterior submarginal rib is adorned by rows of papillae. The proximal border is regularly striated. The apical scales are larger than in form 1 and 2 above, and have a more pointed apex.

Forma gemina was observed only in Malaysia.

Type habitat:

Mallomonas mangofera var. mangofera (Harris & Bradley) Durrschmidt (Figs.)

This organism is distributed world-wide, including tropical habitats (e.g. Takahashi & Hayakawa, 1979).

Reported here from Malaysian Habitat 2, and Lake Fidler and a small farm dam near Springfield, Tasmania. Scales of the Malaysian specimens (Fig.) were very electron-dense.

Mallomonas mangofera f. foveata Durrschmidt (Fig.).

Previously reported only from WHERE? (Durrschmidt, 19) this organism was frequent in Malaysian Habitats 1 and 2. DATE

Mallomonas mangofera f. gracilis morpha (Figs.)

The scales of this organism resemble those of f. gracilis in that there is no well defined anterior submarginal rib, the shield pattern is weakly developed, and the papillae are fused together to form a more or less continuous sec. layer. The scales also have small irregularly shaped pits, mainly along the submarginal area, at the bottom of which are 1-4 circular pores. No typical forma gracilis cells were seen. With respect to scale ornamentation, the specimens stand between M. mangofera var. sulcata Durrschmidt and M. mangofera f. gracilis.

The organism was seen in Malaysian Habitat 2.

Mallomonas insignis Conrad (Figs.)

M. insignis has been reported from Europe, North America, and from New Zealand (Durrschmidt, in press).

Single scales only were observed in Malaysian Sample 2.

Mallomonas morrisonensis Croome & Tyler (Figs.)

Previously reported only from two lakes in Tasmania, Australia (Croome & Tyler, 1983c). Specimens observed in Malaysia differ from the type in having dome-bearing scales only on the anterior part of the cell, and a more variable ornamentation on the scales.

From its LM-appearance M. morrisonensis could well be synonymous with M. tonsurata var. dorsidentata Prowse.

Cells were observed in Malaysian Habitat 2, and Lake Morrison and Sulphide Pool in Tasmania, Australia.

Mallomonas ocellata sp. nov. (Figs.)

Cells are oval or elliptical, with three types of scales: dome-bearing collar scales, rhomboidal body scales and small asymmetrical rear scales.

The body scales have broad submarginal ribs, which are not clearly defined against the shield. The shield is marked with a number of widely spaced, more or less rounded pits. At the bottom of each pit the base-plate is visible, and contains centrally a small, somewhat thickened area, which has one or two pores. Posteriorly on the shield the sec. layer is absent and an area of the base-plate with pores is conspicuous. The proximal part of the posterior submarginal rib is hooded and has a row of pores. The anterior flange is ornamented by a single row of equally spaced papillae. Another one or two rows of papillae adorn the anterior submarginal rib.

The collar scales are depressed triangular. Their domes are extra-ordinary large and square-shaped. One half is delicately perforated, the other is smooth with a conspicuous row of pits similar to those on the shield. Small papillae are scattered over the dome. The shield is ornamented as in the body scales, although the reticulation is more weakly developed. The dorsal submarginal rib is developed like a wing and ends with a sharp pointed thorn, which overtops the distal end of the scale.

The posterior flange has one row of pores running along the edge of the V-rib. The posterior border is smooth. Rear scales are smaller and slightly asymmetric.

The bristles are smooth and taper to a needle-like apex.

Cysts were not observed.

The epithet 'ocellata' refers to the eye-like impression of the pits.

M. ocellata belongs to the section Torquatae Momeu & Péterfi (1979) and shows some resemblance to members of the pumilio group. As in var. pumilio the anterior flanges of the body scales have smooth anterior flanges, ornamented only by rows of papillae. Moreover, regularly spaced depressions are common to both species. In other respects, M. ocellata is clearly distinguished, for instance in the lack of pores on the bottom of each pit, the ornamentation and size of the extraordinary large domes, the shape of the collar scales and the lack of spines on the rear scales.

M. ocellata was common in Malaysian Habitat 2.

Mallomonas papillosa Harris & Bradley (Figs.)

Of world-wide distribution, including localities in the arctic at around 10°C (Asmund & Hilliard, 1961) and Western Australia (Takahashi, 1978).

Reported here from Malaysian Habitats 2 and 3, at temperatures around 28°C.

Mallomonas cf. paxillata (Bradley) Péterfi & Momeu (Figs.)

Only two species are known in which the scales have papillated shields and lack struts on the anterior flanges: M. rasilis Durrschmidt and M. paxillata. The scales reported here appear to be somewhat reduced rear scales of M. paxillata.

The scales were observed from Malaysian Habitat 2.

Mallomonas perforata Hickel & Cronberg (Figs.)

M. perforata is distributed world-wide, with the notable exception of Europe. In Chile it occurs at 10°C, ( ), while in Malaysia it was found at 28°C.

Reported here from Malaysian Samples 1 and 2, and Lake Leake in Tasmania (Feb. 1971).

Mallomonas punctifera Korshikov (Figs.)

This species appears to tolerate a wide range of temperatures: in Germany it occurs at around 4°C, while in Malaysia it inhabits waters at 25-30°C.

Reported here from Malaysian Habitat 2.

Mallomonas splendens (G.S. West) Playfair emend. Croome, Durrschmidt & Tyler (Figs.)

This species is of frequent occurrence in Australian waters. Besides several LM observations from Tasmania, Victoria and Queensland, EM-verified records exist from habitats in the Northern Territory and New South Wales (Croome et al., in press). Other records exist from Holland (Conrad, 1933), Java (see Huber-Pestalozzi, 1941) and India (Philipose, 1953).

Forma splendens was observed in Malaysian Habitats 1 and 2, and in Woodford Creek Reservoir in New South Wales and Umbungbung and Kulukuluku Billabongs in the Northern Territory, Australia.

Mallomonas splendens f. arnhemensis Croome, Durrschmidt & Tyler (Figs.)

This forma, which differs from the type in possessing a large depression in the body scales, is reported here from Malaysian Habitats NUMBERS , and from Umbungbung and Kulukuluku Billabongs, Northern Territory of Australia.

Mallomonas tonsurata Teiling var. tonsurata (Figs.)

Of world-wide distribution, including tropical loaclities (e.g. Compère, 1975).

Reported here from Malaysian Habitat 2, and Lake Hume on the border of New South Wales and Victoria, Australia (Sept. 1984).

Mallomonas tropica sp. nov. (Figs.)

syn.: M. paxillata in Takahashi & Hayakawa (1979), p.131, fig. 25.
Cells oval or broad elliptical, with tripartite scales and unilaterally serrated bristles. Body scales are oval, the shield and dome bearing regularly spaced papillae which are arranged in more or less longitudinal or somewhat oblique rows. The domes are small and rounded at their distal ends. One half is minutely perforated. The dome is not clearly delimited from the shield and only the median part is conspicuously vaulted. The proximal area of the shield reveals a small circular pore, right in the angle of the "V". The V-rib has radial struts and a smooth hood. The rear part of the anterior flange reveals short struts radiating from the anterior submarginal rib, while the front part is very much reduced. The anterior submarginal rib is present only as a smooth, slightly thickened rib, the prolongation of which surrounds the dome. The posterior flange is smooth and the proximal border striated.

The apical scales deviate slightly from the body scales in that they are smaller, slightly asymmetric and have relatively large domes. The anterior flange and the prolongation of the anterior submarginal rib are more clearly developed than in the body scales.

The bristles are long, slightly curved and unilaterally serrated, and terminate in a pointed tip.

Cysts were not observed.

M. tropica belongs to ser. Papillosae Momeu & Peterfi of the sect. Mallomonas (Momeu & Peterfi, 1979). It bears close resemblance to M. papillosa particularly in having papillated shields and serrated bristles. The distinctive characteristics of M. tropica are the reduced anterior flanges, which lack radial struts on the foremost part and possess only vestiges of these on the rear part, the rudimentarily developed anterior submarginal rib, the significantly larger scales, and the denser papillation of the shield. In addition, the posterior submarginal rib and the proximal border are striated. The latter feature is present in M. rasilis, but this species lacks radial struts completely and has no anterior submarginal rib. There are also some similarities with M. paxillata, e.g. the dense papillation of the shield and the small domes, but in other respects, e.g. the serrated bristles, the peck-like projection at one side of the dome and the striated V-rib, they are different.

M. tropica was observed in Malaysian Habitat

Mallomonas sp. 1 (Fig.)

This scale has a similar alveolar pattern as M. adamas or M. allorgei Conrad. The V-rib is situated in the anterior half of the scale and there is a stout beak-shaped process. Observed in Malaysian Habitat NJP'BLK.

Mallomonas sp. 2 (Fig.)

Several scales were found with this distinct hexagonal pattern, in Malaysian Habitat .

Mallomonas sp. 3 (Fig.)

These scales belong to a closely related species of M. bronchartiana. Observed in Malaysian Habitat .

Mallomonas sp. 4 (Fig.)

This body scale bears papillae similar to those of M. mangofera, and has a mesh-pattern similar to that of M. favosa, in addition to a ribbed posterior flange, a striated posterior submarginal rib, and a striated proximal border. Observed in Malaysian Habitat .

Synura curtispina (Petersen & Hansen) Asmund (Figs.)

Of world-wide occurrence, including tropical localities (e.g. Takahashi & Hayakawa, 1979).

Reported here from Malaysian Habitats 1 and 2, and Lake Leake and Lake Fidler in Tasmania, Lake Hume on the border of New South Wales and Victoria, and Kulukuluku Billabong, Northern Territory of Australia.

Synura echinulata Korshikov (Figs.)

Widely distributed, including tropical localities (e.g. Compère, 1975).

Reported here from Malaysian Habitats 1 and 2, and Kulukuluku Billabong, Northern Territory of Australia.

Synura petersenii Korshikov (Figs.)

One of the most frequently reported Mallomonadacean species in the world, it is also known from the tropics (e.g. Compère, 1975; Takahashi, 1978).

S. petersenii was frequent in Malaysian Habitats 1 and 2, and in Australia was found in Lake Morrison, Lake Fidler, Risdon Brook Dam, Arthurs Lake, Woods Lake, Lake Leake and the Macquarie River in Tasmania, Kulukuluku and Umbungbung Billabongs in the Northern Territory, Lake Hume on the border of New South Wales and Victoria, and Gumeracha Weir on the Torrens River in South Australia.

Synura sphagnicola Korshikov (Figs.)

Of world-wide distribution, S. sphagnicola occurs mainly in acid and humic waters (

Reported here from Malaysian Habitat 1.

Synura spinosa Korshikov (Figs.)

Of world-wide distribution, S. spinosa is reported here from Malaysian Habitats 2 and 3, and Lake Hume on the border of New South Wales and Victoria.

Chrysodidymus synuroides Prowse (Fig.)

Syn.: Synura microcrepis Nygaard (1978).
This species was originally described from Malaysia. It is characterized by two-celled colonies, and its scales have been reported world-wide: Greenland (Nygaard, 1978), Chile (Durrschmidt, 1982), Japan (Takahashi, 1978), North America (e.g. Puytorac et al., 1972). The Malaysian specimens lack the small additional layer on the posterior part of the scales, which may or may not be present in this taxon.

C. synuroides was frequent in all three Malaysian Habitats, but was not observed in Australia.

Chrysosphaerella astrea sp. nov. (Figs.)

Cells solitary, spherical, covered with about 10-15 radially directing spine scales and numerous elliptical plate scales. The spines of the spine scales are straight, hollow and taper to a very small, but not pointed apex. The tip is bifurcate, although this is visible only

at higher magnification. The bobbin-like base is composed of almost equally sized plates. The basal disc and the second disc are separated by a small isthmus. The plate scales are elliptical, each with a smooth outer margin and a wavy edged central area.

Cysts were not observed.

The epithet refers to the star-like appearance of the cells. C. astrea belongs, together with C. coronacircumspina Wujek & Kristiansen and C. salina Birch-Andersen, to the non-colonial species of the genus Chrysosphaerella,. C. astrea is distinguished from all other Chrysosphaerella species by the shape of the spine scales, the equally long spines, the regularly sized or sometimes slightly larger basal disc, and the small isthmus. With respect to the spine scales C. astrea represents a transitional form between C. coronacircumspina and C. brevispina. The spines are similar to those specimens of C. coronacircumspina published by Preisig & Takahashi (1978) as C. solitaria.

C. astrea was observed in Malaysian Habitat .

Chromophysomonas trioralis (Takahashi) Preisig & Hibberd (Figs.)

Of world-wide occurrence, C. trioralis has not previously been reported from the tropics.

Reported here from Malaysian Habitat 2, and Lake Fidler, Lake Morrison and Tooms Lake in Tasmania.

Paraphysomonas vestita (Stokes) de Saedeleer (Figs.)

Of world-wide occurrence, including tropical localities (e.g. Compère, 1975).

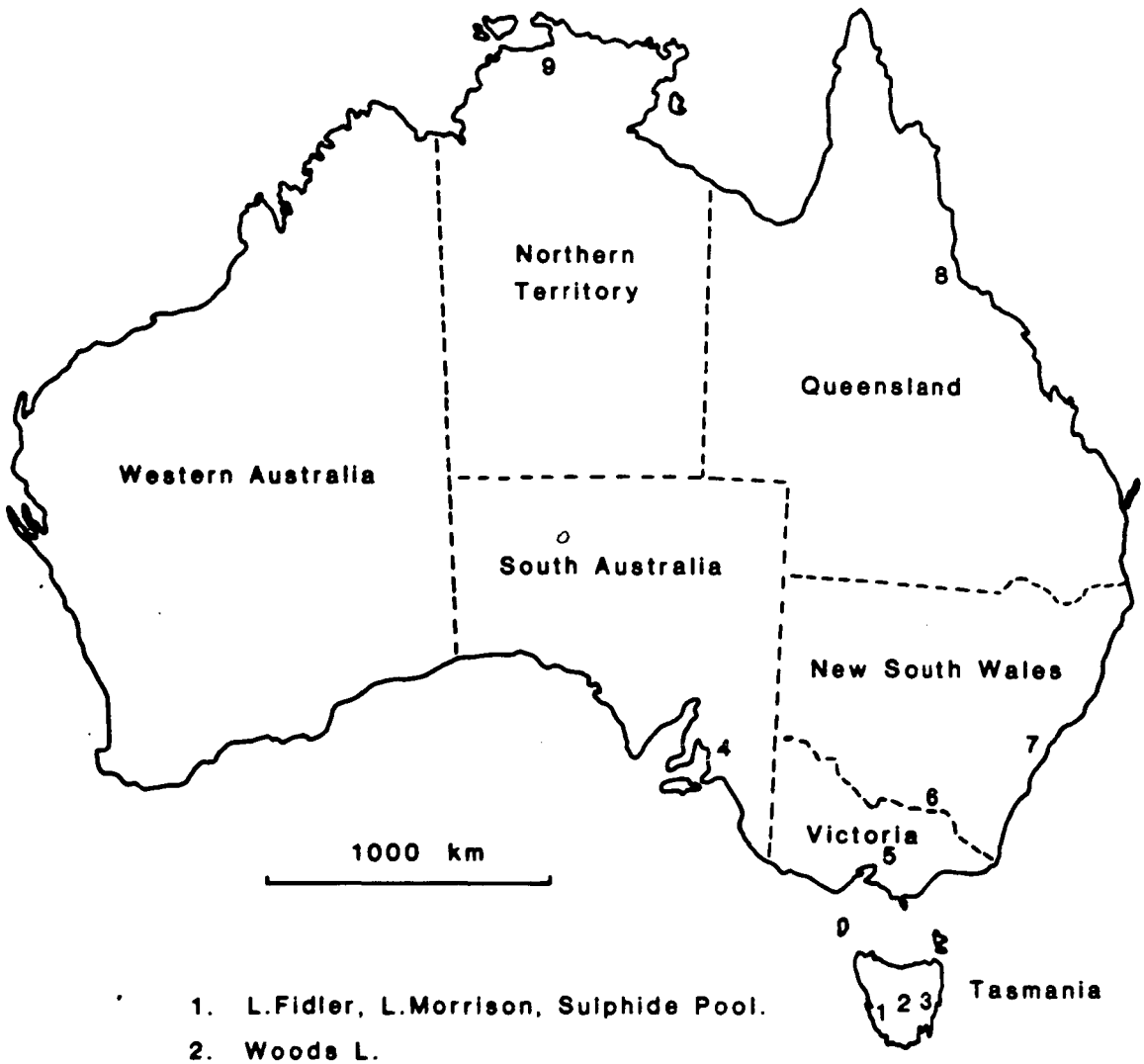
Reported here from Malaysian Habitat 2, and Lake Fidler, Lake Morrison, Sulphide Pool and a small farm dam in Tasmania, and Gumeracha Weir on the River Torrens in South Australia.

Discussion

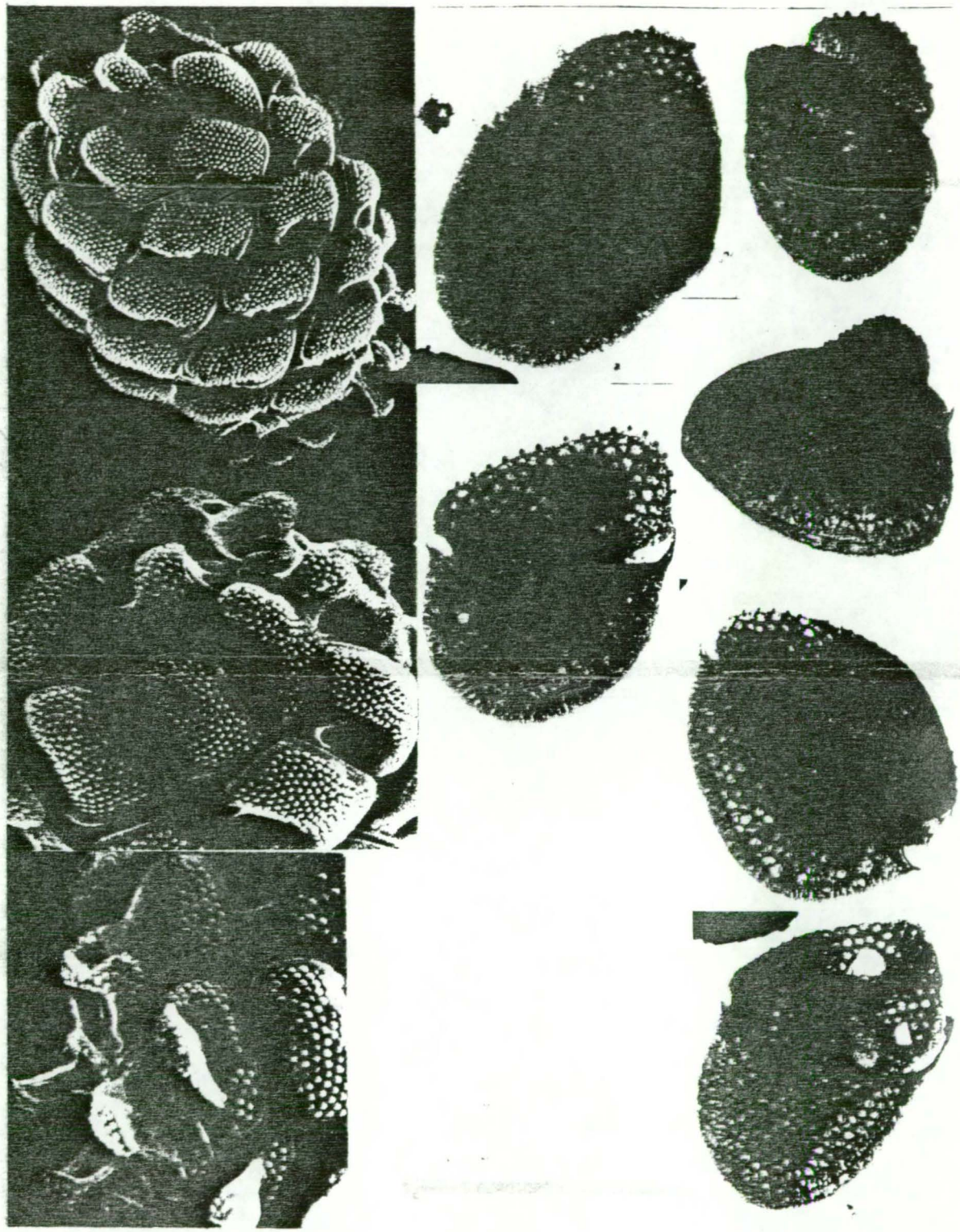
The observations herein show that Malaysia and Australia are relatively rich in Chrysophytes; twenty nine taxa of Mallomonadaceae are reported from Malaysia, and seventeen from Australia. Most of the observations are new geographical records. This is particularly so for Malaysia, where the only previous report of the family was by light microscopy (Prowse, 1962). Such observations are no longer acceptable in taxonomic studies of the Mallomonadaceae, and in this regard it is interesting that although Prowse reported Synura uvella (Stein.) Korsh. from Malaysia, we were unable to find this organism in either Malaysia or Australia, finding instead several other members of this genus.

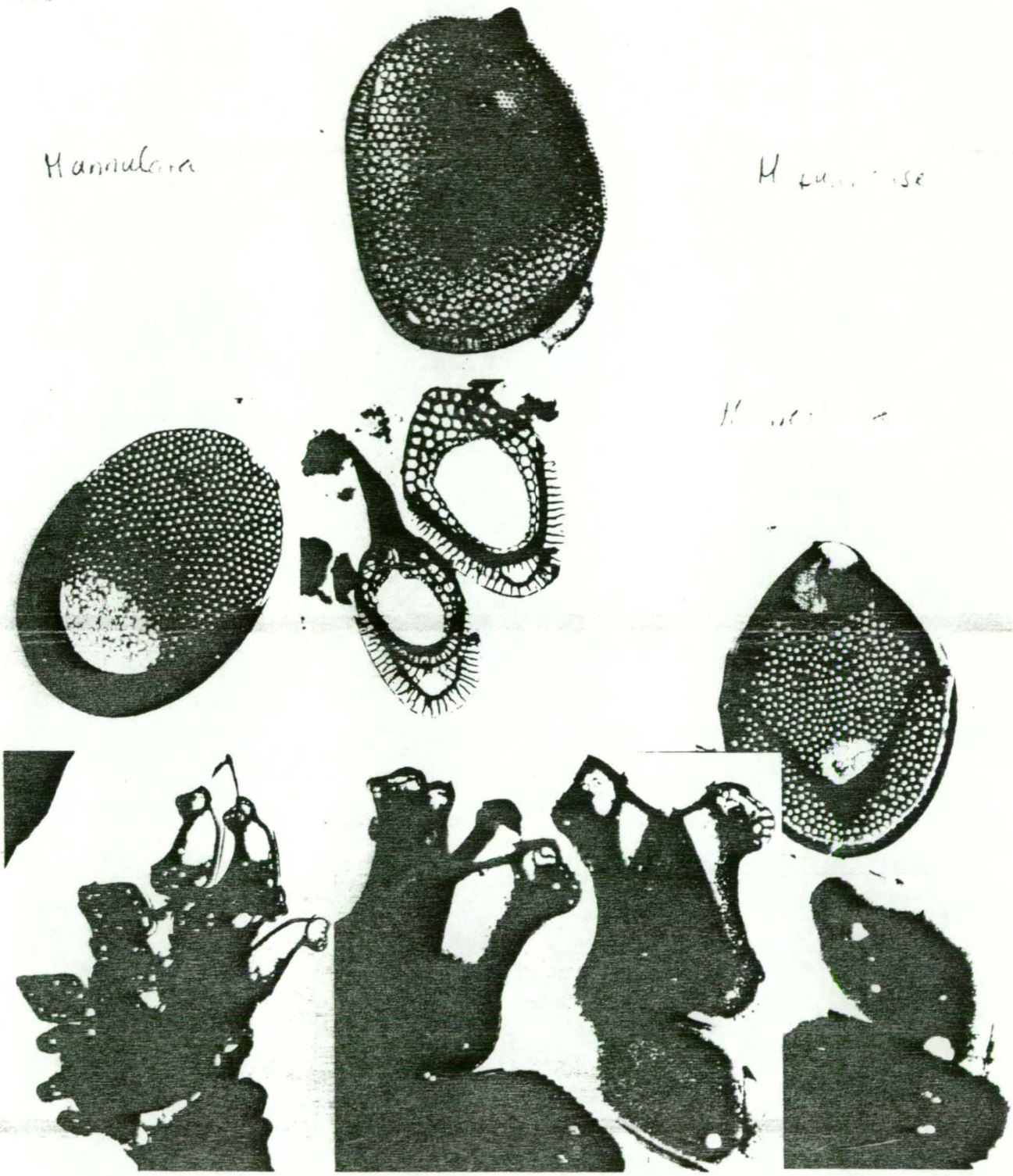
Some nineteen of the taxa reported herein are common and widely distributed in those other parts of the world where Chrysophytes have been studied. Others, e.g. Mallomonas adamas, M. splendens, and M. favosa, are of rare occurrence world-wide, but appeared frequently in our samples. Twelve of the species found in Malaysia have been reported previously only from temperate zones, and three of these, Mallomonas annulata, M. striata and M. papillosa, were previously considered to be stenothermal with oligothermal tendencies ( ). The observations herein from Malaysia, and tropical areas of Australia, support the view of Kristiansen (1981) that the Chrysophyceae can no longer be regarded as basically cold water organisms.

Australia is a large land-mass, with an area of some 7.7×10^6 km^2. It stretches from tropical areas at around 12°S to areas of cool-temperate climate around 42°S. Much of Australia is arid and most of its standing waters are adjacent to its coasts, and on the island State of Tasmania. Samples from many Australian water bodies were examined during this study: the most significant collecting sites for members of the Mallomonadaceae are shown in Figure .



1. L.Fidler, L.Morrison, Sulphide Pool.
2. Woods L.
3. L.Leake, Tooms L.
4. Gumeracha Weir.
5. Yan Yean Res.
6. L.Hume.
7. Woodford Ck. Res.
8. Ross River.
9. Kulukuluku & Umbungbung Billabongs.





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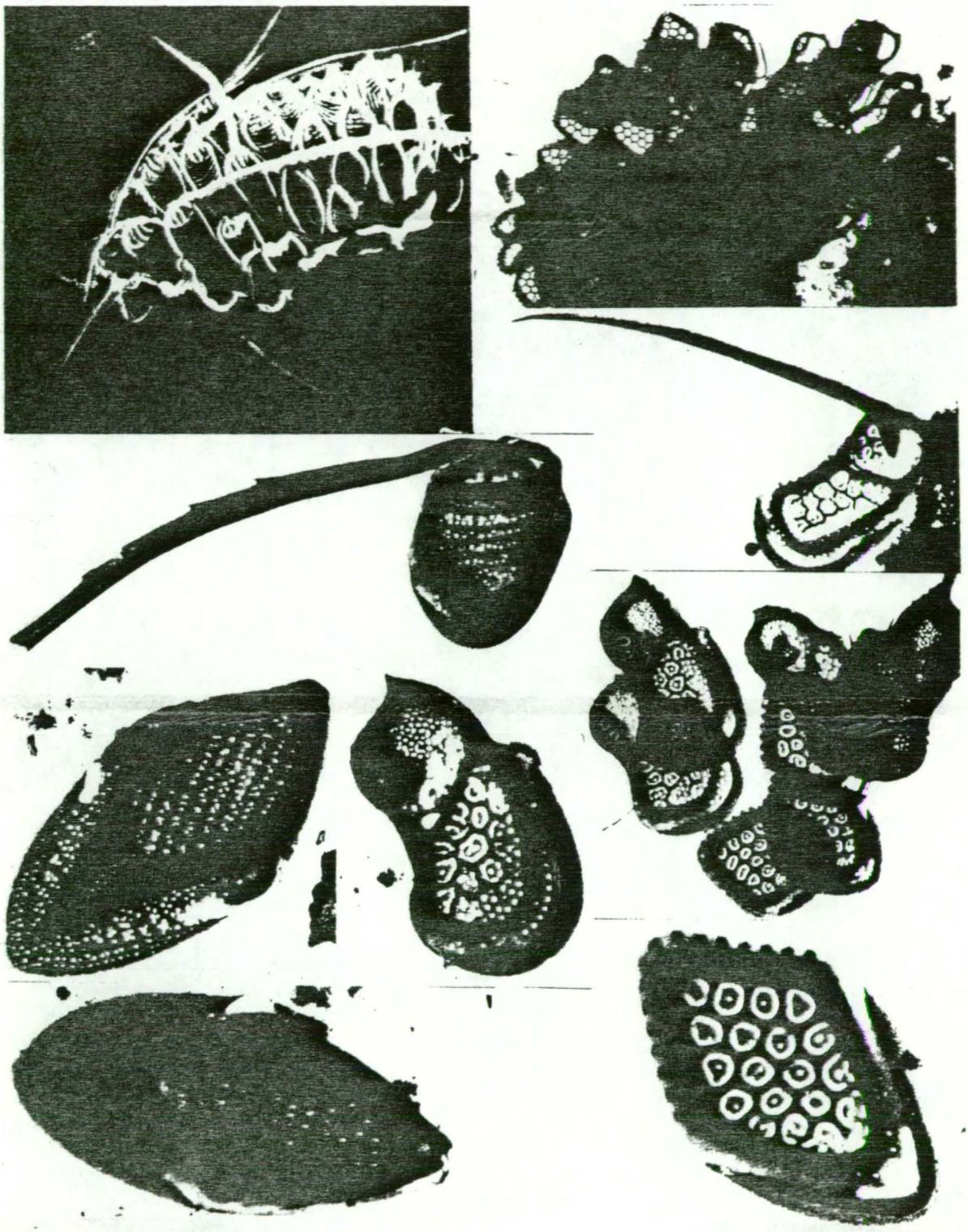

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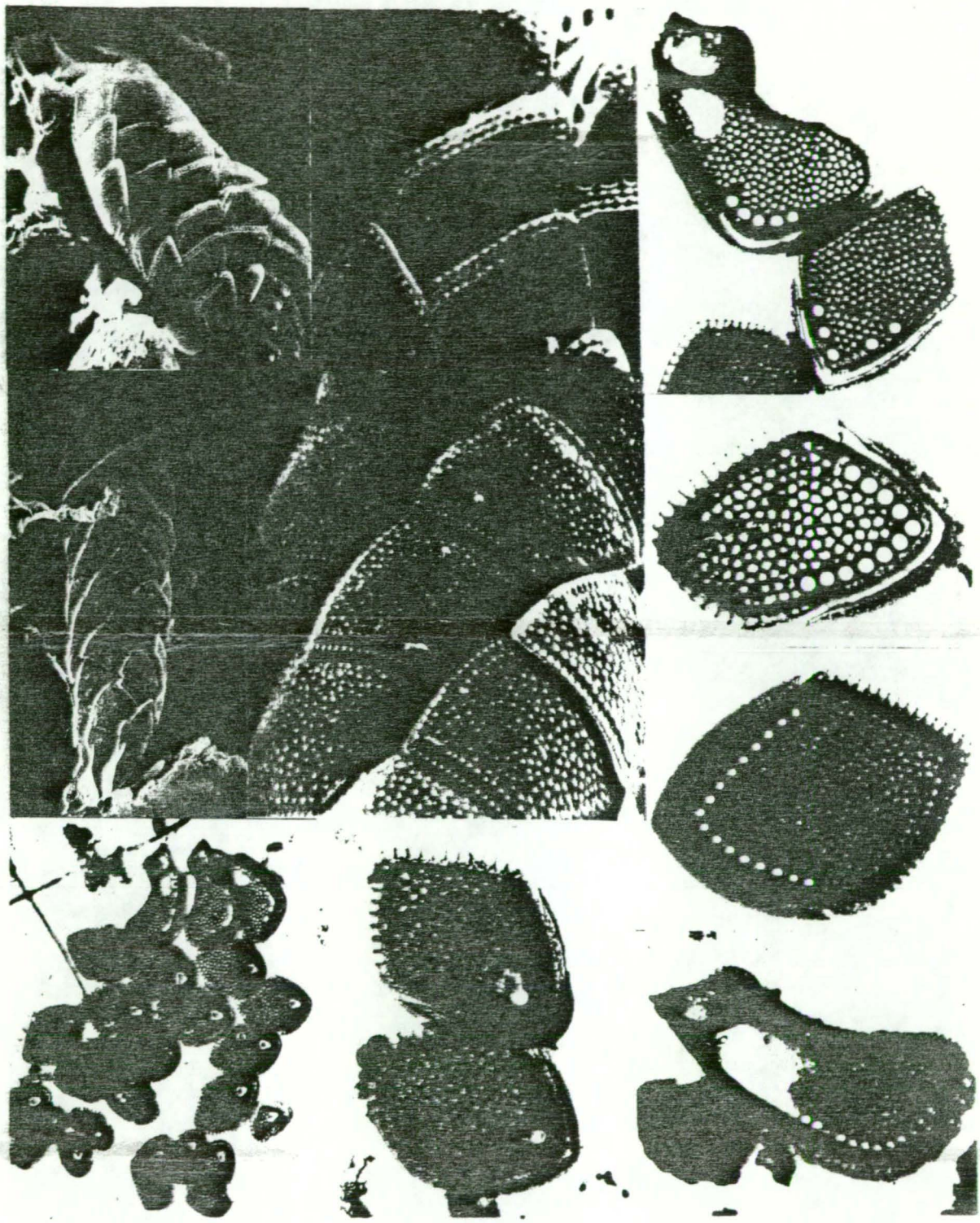

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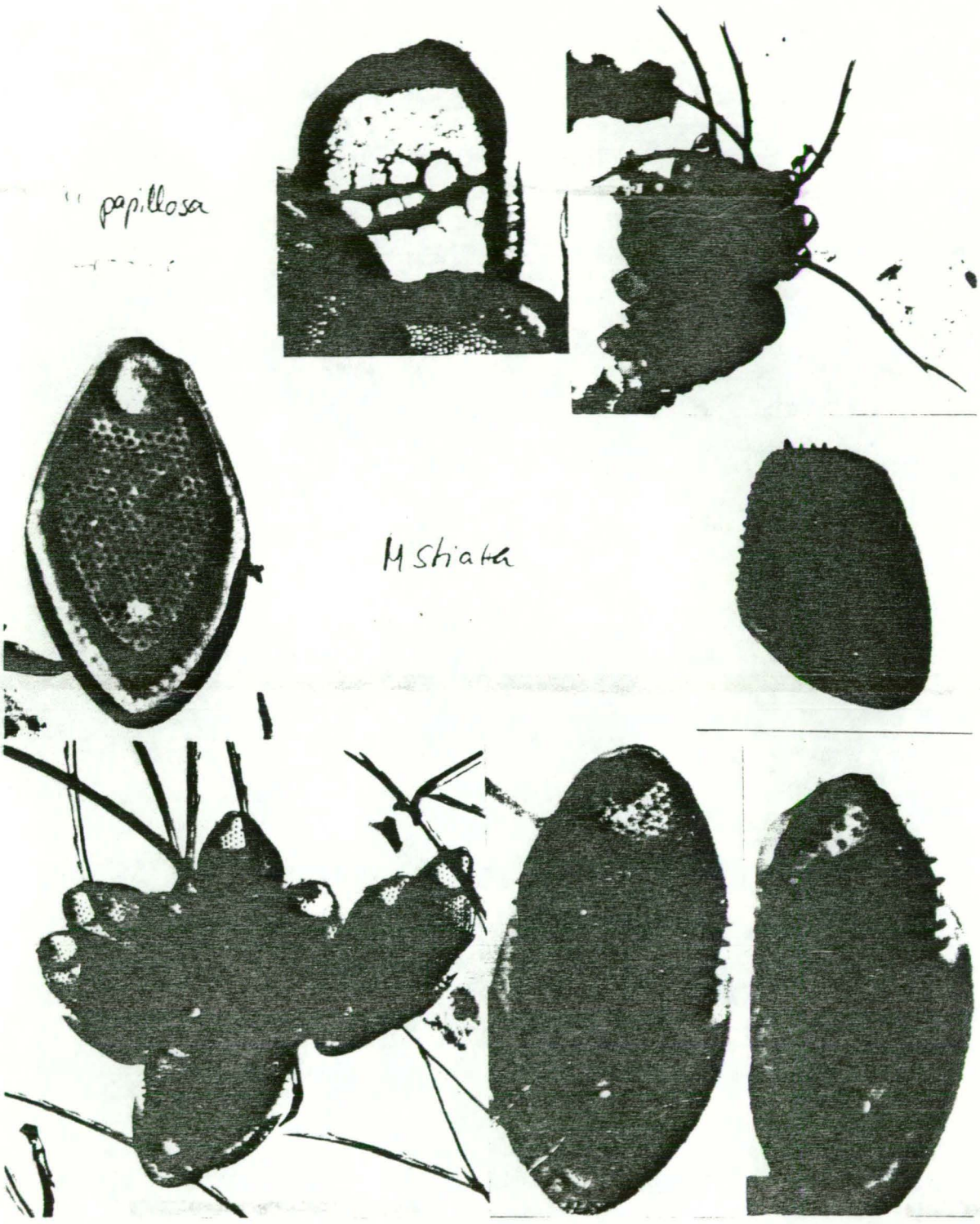

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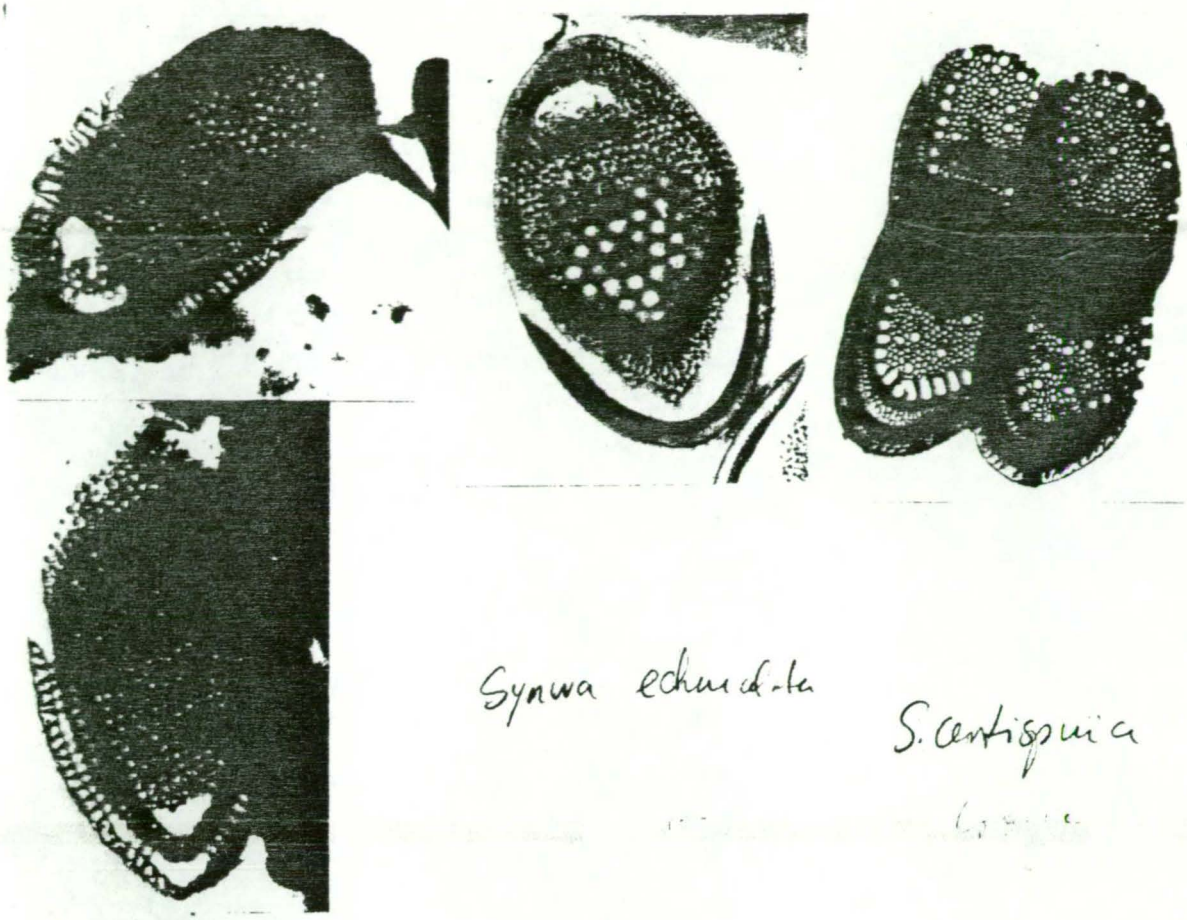
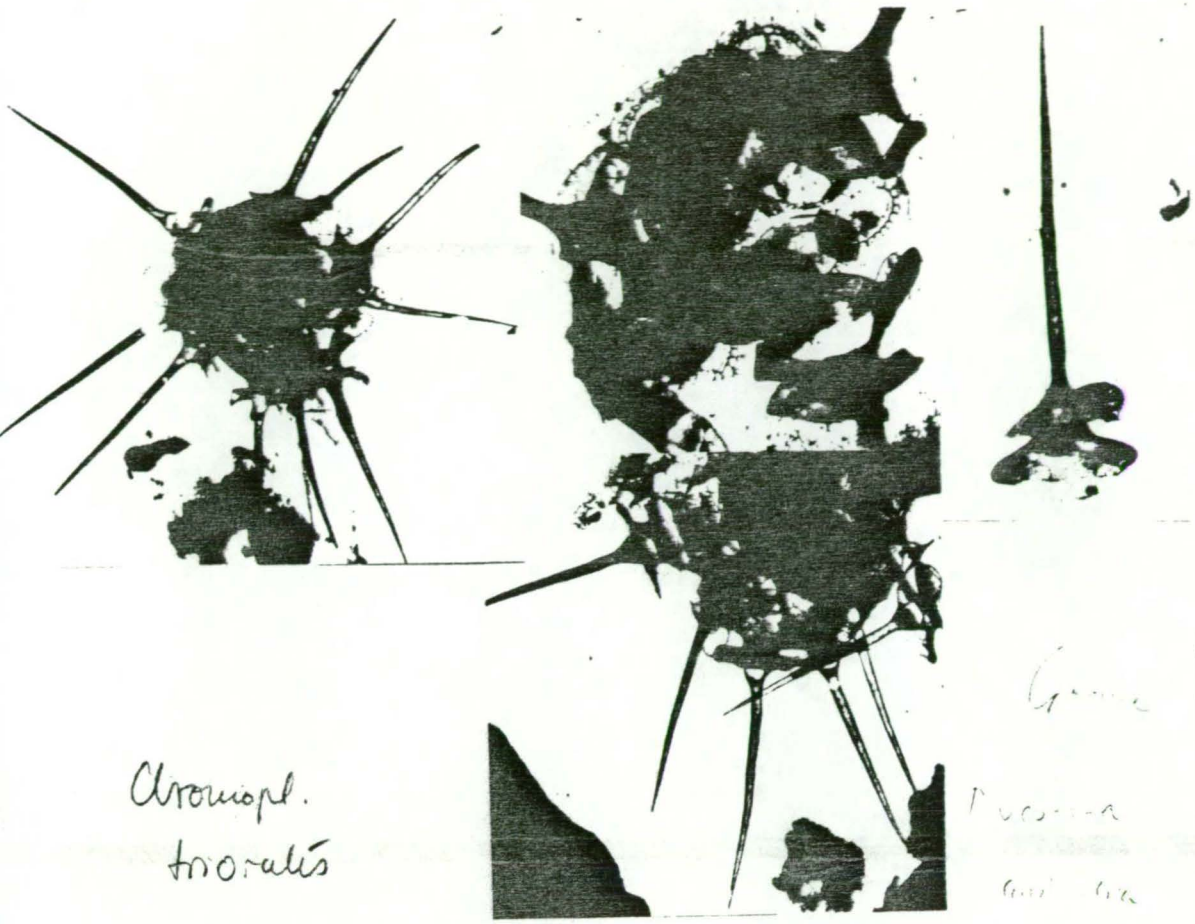


Synura echinulata

S. centispina

S. petersenii

VII



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Appendix IX

CROOME, R.L. and TYLER, P.A. (1984d). Australian Mallomonadaceae (Chrysophyceae). NORDIC JOURNAL OF BOTANY (Submitted).

Australian Mallomonadaceae (Chrysophyceae)

by

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Abstract

Twenty eight species of Mallomonadaceae (Chrysophyceae) of the genera Chromophysomonas, Chrysosphaerella, Mallomonas, Paraphysomonas and Synura are recognized by electron microscopy, from a wide range of Australian freshwater habitats, from tropical to temperate regions, and from a range of lake trophic types. While most are of worldwide occurrence, several species are as yet known only from Australia. Several highly dystrophic lakes in Tasmania are particularly rich in species numbers and in population densities.

Introduction

Among the sixty or more taxonomic papers on Australian freshwater algae some indicate the presence of Chrysophytes in many parts of the country. However, since most of these works pre-date the electron microscope most specific names cannot be accepted. Transmission and scanning electron microscopy has now been employed in the study of Australia's rich Chrysophyte flora. Takahashi (1978) reported three taxa from Western Australia and six individual species have been described by Croome & Tyler (1983a-c, 1984) and Croome et al. (1984). Recently those Australian species which also occur in Malaysia have been illustrated (Croome & Durrschmidt, 1984). In this paper we review the 28 species of Australian Mallomonadaceae which have been studied by electron microscopy, reporting and figuring several species for the first time from this country.

Materials and Methods

Samples were collected with a 25 µm pore plankton net and fixed with formalin. Specimens for scanning electron microscopy (SEM) were placed on polylysine-coated glass coverslips (Marchant & Thomas, 1983), dehydrated in an acetone series, critical point dried using CO_2 as the intermediary fluid, sputter-coated with gold, and viewed using a Philips 505 SEM. Material for transmission electron microscopy (TEM) was put onto Formvar-coated copper grids, shadowed with platinum/palladium (2 parts to 1) and viewed using an Hitachi H-300 TEM.

Survey of Species

The main collecting sites are shown in Figure 1. Some of the 27 Australian species positively identified by us by electron microscopy, listed below, have been illustrated by Croome & Durrschmidt (1984). The remainder are illustrated in this paper.

Chromophysomonas trioralis (Takahashi) Preisig & Hibberd

Lake Fidler, Lake Morrison, Tooms Lake, Tasmania.

Chrysophaerella coronacircumspina Wujek & Kristiansen (Fig. 2).

Lake Fidler, Lake Morrison, Tasmania.

Chrysosphaerella brevispina Korshikov emend. Harris & Bradley (Fig. 3).

Lake Morrison, Tasmania.

Mallomonas adamas Harris & Bradley emend. Durrschmidt & Croome

Lake Fidler, Lake Rosebery, Tasmania. The Australian and Malaysian records (Durrschmidt & Croome,1984) are the first since its original description from England (Harris & Bradley, 1960).

Mallomonas akrokomos Ruttner (Figs. 4-5).

Lake Leake, Tasmania; Gumeracha Weir, South Australia.

Mallomonas annulata (Harris & Bradley) Harris

Gumeracha Weir, South Australia; Lake Leake, Tasmania.

Mallomonas areolata Nygaard

Lake Morrison, Tasmania.

Mallomonas cf. areolata Nygaard (Fig. 6).

Gumeracha Weir, South Australia. Only one scale seen in the sample, and this record is tentative.

Mallomonas calceolus Bradley (Figs. 7-8).

Sulphide Pool, Tasmania.

Mallomonas elliptica Matvienko

Kulukuluku and Umbungbung Billabongs, Northern Territory. The bristles of our Australian forms are unusually long, up to 60 μm (Croome & Tyler, 1983b).

Mallomonas favosa Nicholls

Kulukuluku Billabong, Northern Territory.

Mallomonas lychenensis Conrad (Fig. 9).

Sulphide Pool, Tasmania. This organism is probably a forma of M. lychenensis but further TEM observations are desirable.

Mallomonas mangofera Harris & Bradley

Lake Fidler and farm dam nr. Springfield, Tasmania.

Mallomonas morrisonensis Croome & Tyler

Lake Morrison, Sulphide Pool, Lake Rosebery, and Lake Chisholm, Tasmania.

Mallomonas papillosa Harris & Bradley .

Recorded by Takahashi (1978) from Western Australia.

Mallomonas perforata Hickel & Cronberg

Lake Leake, Tasmania.

Mallomonas plumosa Croome & Tyler (Figs. 10-13)

Lake Leake, Tooms Lake, Tasmania; Lake Hume, Victoria/New South Wales; Yan Yean, Victoria. This distinctive species, readily identified by light microscopy, also occurs in New Zealand (Durrschmidt, pers. comm.).

Mallomonas pseudocratis Durrschmidt (Fig. 14)

Lake Leake, Tasmania.

Mallomonas splendens (G.S. West) Playfair emend. Croome, Durrschmidt & Tyler.

Two formae of this species are recognized (Croome, Durrschmidt & Tyler, 1984).

f. splendens: Woodford Creek Reservoir, New South Wales; Umbungbung and Kulukuluku Billabongs, Northern Territory

f. arnhemensis: Kulukuluku and Umbungbung Billabongs, Northern Territory.

By light microscopy only, M. splendens has also been observed in Yan Yean Reservoir, Victoria, Lake Fidler, Tasmania and Ross River Reservoir, Queensland.

Mallomonas striata var. serrata Harris & Bradley (Fig. 15)

Woods Lake, Tasmania. Takahashi (1978) recorded M. striata from Western Australia.

Mallomonas tasmanica Croome & Tyler (Fig. 16)

Sulphide Pool, Tasmania.

Mallomonas tonsurata Teiling

Lake Hume, Victoria/New South Wales.

Paraphysomonas caelifrica Preisig & Hibberd (Figs. 17-19).

Sulphide Pool, Tasmania. The cells have shorter spines than those depicted by Preisig & Hibberd (1982) but Preisig (pers. comm.) considers this feature to be variable.

Paraphysomonas vestita (Stokes) de Saedeleer

Lake Fidler, Lake Morrison, Sulphide Pool, Lake Rosebery, farm dam nr. Springfield, Tasmania; Gumeracha Weir, South Australia. Takahashi (1978) recorded this species from Western Australia.

Synura australiensis Playfair emend. Croome & Tyler (Figs. 20-21).

Lake Leake, Tasmania; Lake Hume, Victoria/New South Wales; Kulukuluku, Northern Territory.

In its cell morphology S. australiensis is a distinctive species, with cells 34-70 μm x 6.0-7.5 μm. However, its scales are very similar to those of S. petersenii except that they are considerably more elongate. The possible relationship between the two is discussed by Croome & Tyler (1984).

Synura curtispina (Petersen & Hansen) Asmund.

Lake Leake, Lake Fidler, Tasmania; Lake Hume, Victoria/New South Wales; Kulukuluku Billabong, Northern Territory.

Synura echinulata Korshikov

Kulukuluku Billabong, Northern Territory.

Synura petersenii Korshikov

Lake Morrison, Lake Fidler, Risdon Brook Dam, Arthurs Lake, Woods Lake, Lake Leake, Macquarie River at Ross, Tasmania; Kulukuluku and Umbungbung Billabongs, Northern Territory; Lake Hume, Victoria/New South Wales; Gumeracha Weir, South Australia.

Synura spinosa Korshikov

Lake Rosebery, Tasmania; Lake Hume, Victoria/New South Wales.

Discussion

The total number of species of Mallomonadaceae ~~now~~ recorded from Australia now stands at 28. Though, undoubtedly, more Chrysophytes will be found, and the range of others extended by further study, we have examined many dozens of samples from across Australia and believe many species to be sporadic and disjunct in their distribution. We have found them right across the continent from the tropical north to cool temperate Tasmania, and from a range of lake types spanning oligotrophic, eutrophic, dystrophic, clear and turbid. The fact that 7 species were recorded from the tropical Northern Territory is further evidence that the Chrysophyceae can no longer be regarded as a cold water group (Kristiansen, 1981).

Chrysophytes are known to favour dystrophic waters (e.g. Ilmavirta, 1983) and it is in such waters in Australia that the richest array of species occurs. The Gordon River lakes in south-west Tasmania (Morrison, Fidler and Sulphide Pool) are highly dystrophic and contain 14 species, 50% of the recorded Australian flora. Eight species were found only in these dystrophic lakes. In these lakes they occur in high numbers, frequently reaching bloom proportions. There is evidence that they select intermediate depths in the water column by day and disperse at night (Croome & Tyler, 1983b). Of particular note is the genus Paraphysomonas, usually found sporadically in small numbers. In Sulphide Pool, P. caelifrica has been observed at concentrations of up to 500 cells per mL.

Most Australian species are worldwide in their distribution. However, Synura australiensis (Croome & Tyler, 1984) is yet to be found outside Australia, M. tasmanica (Croome & Tyler, 1983b) appears to be restricted to Tasmania, and M. plumosa (Croome & Tyler, 1983a), perhaps the most distinctive species yet described, to Australia and New Zealand.

Acknowledgements

We thank the Australian Research Grants Scheme and the University of Tasmania for research grants, and Dr. D.P. Thomas and Mr. A. Eastgate for assistance with specimen preparation and operation of electron microscopes. Samples from the Northern Territory were collected while carrying out limnological surveys under contract to the Office of the Supervising Scientist for the Alligator Rivers Region. The light micrograph of Synura australiensis (Fig. 20) was kindly supplied by Dr. Hau U Ling.

References

CROOME, R.L., DURRSCHMIDT, M. & TYLER, P.A. 1984. A light and electron microscopical investigation of Mallomonas splendens (West) Playfair. - Plant Systematics and Evolution (in press).

CROOME, R.L. & TYLER, P.A. 1983a. Mallomonas plumosa (Chrysophyceae) a new species from Australia. - Br. phycol. J. 18: 151-158.

CROOME, R.L. & TYLER, P.A. 1983b. Mallomonopsis tasmanica sp. nov. (Chrysophyceae) and Mallomonopsis elliptica Matvienko from Australia. - Br. phycol. J. 18: 357-365.

CROOME, R.L. & TYLER, P.A. 1983c. Mallomonas morrisonensis (Chrysophyceae) a new species from Australia. - Br. phycol. J. 18: 383-389.

CROOME, R.L. & TYLER, P.A. 1984. A re-examination of Playfair's Synura australiensis and Synura granulosa. - Nordic J. Bot. (submitted).

DURRSCHMIDT, M. & CROOME, R.L. 1984. Contribution to the knowledge of Mallomonadaceae (Chrysophyceae) from Malaysia and Australia. - Nordic J. Bot. (submitted).

HARRIS, K. & BRADLEY, D.E. 1960. A taxonomic study of Mallomonas. - J. gen. Microbiol. 22: 750-777.

ILMAVIRTA, V. 1983. The role of flagellated phytoplankton in chains of small brown-water lakes in southern Finland. - Ann. Bot. Fennici 20: 187-195.

KRISTIANSEN, J. 1981. Distribution problems in the Synuraceae (Chrysophyceae). - Verh. int. Verein. theor. angew. Limnol. 21: 1444-1448.

MARCHANT, H.J. & THOMAS, D.P. 1983. Polylysine as an adhesive for the attachment of nanoplankton to substrates for electron microscopy. - J. micros. 131: 127-129.

PREISIG, H.R. & HIBBERD, D.J. 1982. Ultrastructure and taxonomy of Paraphysomonas (Chrysophyceae) and related genera I. - Nordic. J. Bot. 2: 397-420.

TAKAHASHI, E. 1978. Electron microscopical studies on the Synuraceae (Chrysophyceae) in Japan. - Tokai Univ. Press, Tokyo.

Figure Captions

Fig. 1. Map showing location of principal collecting sites.

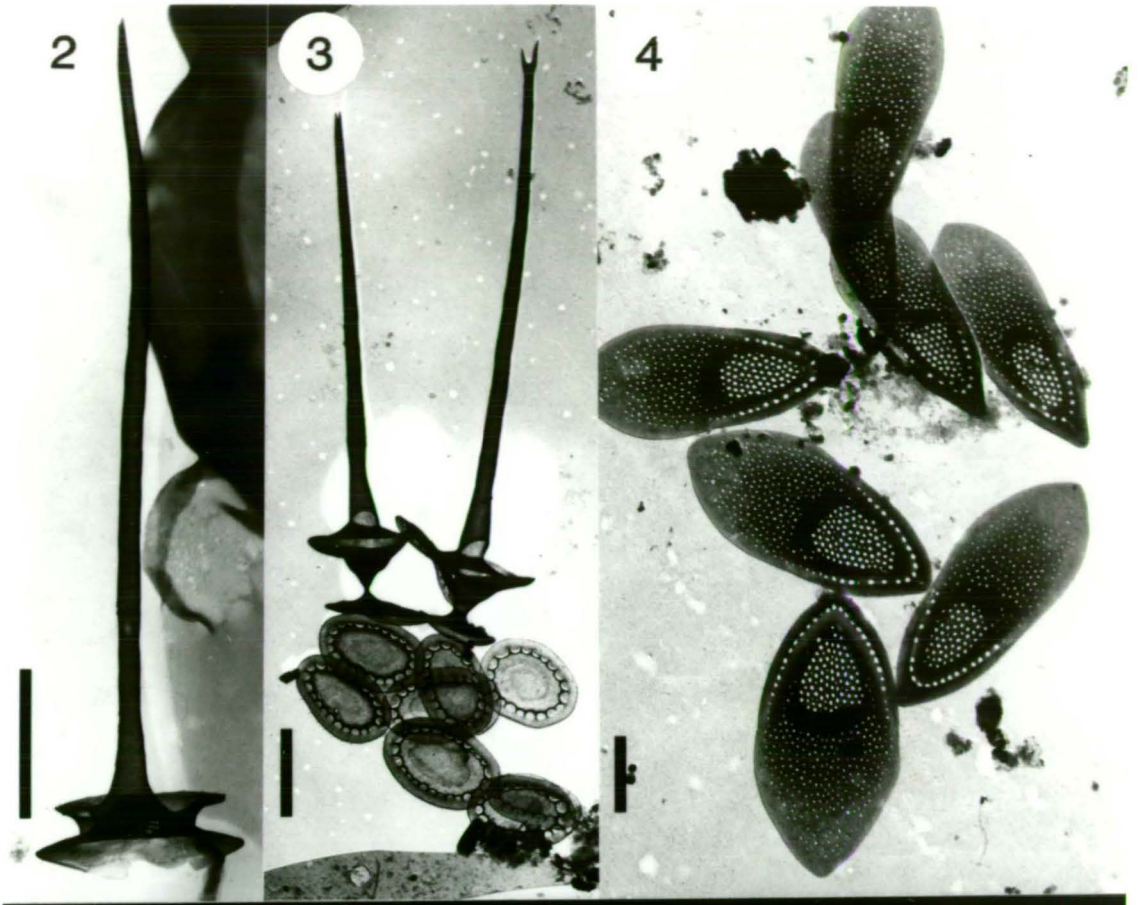
Fig. 2. Chrysosphaerella coronacircumspina. TEM of isolated spine. Scale bar denotes 2 μm. Fig. 3. Chrysosphaerella brevispina. TEM of spines and scales. Scale bar denotes 2 μm. Fig. 4. Mallomonas akrokomos. TEM of body scales. Scale bar denotes 1 μm. Fig. 5. Mallomonas akrokomos. SEM of whole cell. Scale bar denotes 5 μm.

Fig. 6. cf. Mallomonas areolata. TEM of scale with bristle. Scale bar denotes 2 μm. Figs. 7 & 8. Mallomonas calceolus. TEM of scales. Scale bar denotes 1 μm. Fig. 9. Mallomonas lychenensis. SEM of whole cell. Scale bar denotes 5 μm.

Figs. 10-13. Mallomonas plumosa. Fig. 10. SEM of whole cell. The bristles were lost during fixation. Scale bar denotes 5 μm. Fig. 11. TEM of body scale. Scale bar denotes 1 μm. Fig. 12. TEM of plume-bristle from an apical scale. Scale bar denotes 1 μm. Fig. 13. TEM of bristle from a body scale. Scale bar denotes 5 μm.

Fig. 14. Mallomonas pseudocratis. SEM of whole cell. Scale bar denotes 10 μm. Fig. 15. Mallomonas striata var. serrata. SEM of whole cell. Scale bar denotes 10 μm. Fig. 16. Mallomonas tasmanica. TEM of scale and bristles. Scale bar denotes 1 μm.

Figs. 17-19. Paraphysomonas caelifrica. Fig. 17. TEM of whole cell. Scale bar denotes 1 μm. Fig. 18. TEM of scales and spine. Scale bar denotes 0.5 μm. Fig. 19. TEM of single scale. Scale bar denotes 0.5 μm. Figs. 20-21. Synura australiensis. Fig. 20. Light micrograph of colony (a negative print). Scale bar denotes 20 μm. Fig. 21. TEM of scale. Scale bar denotes 1 μm.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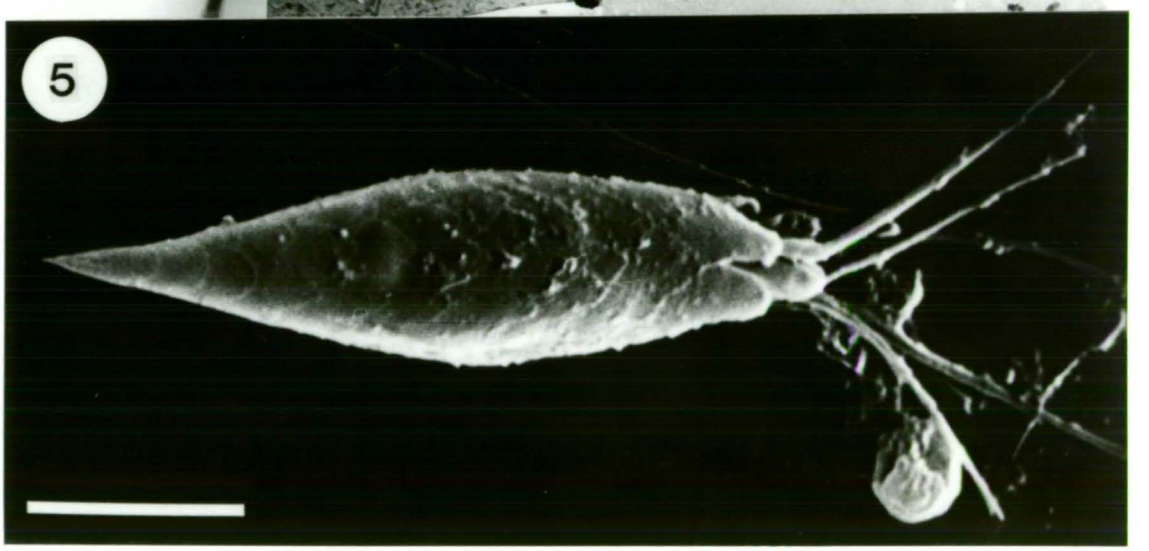
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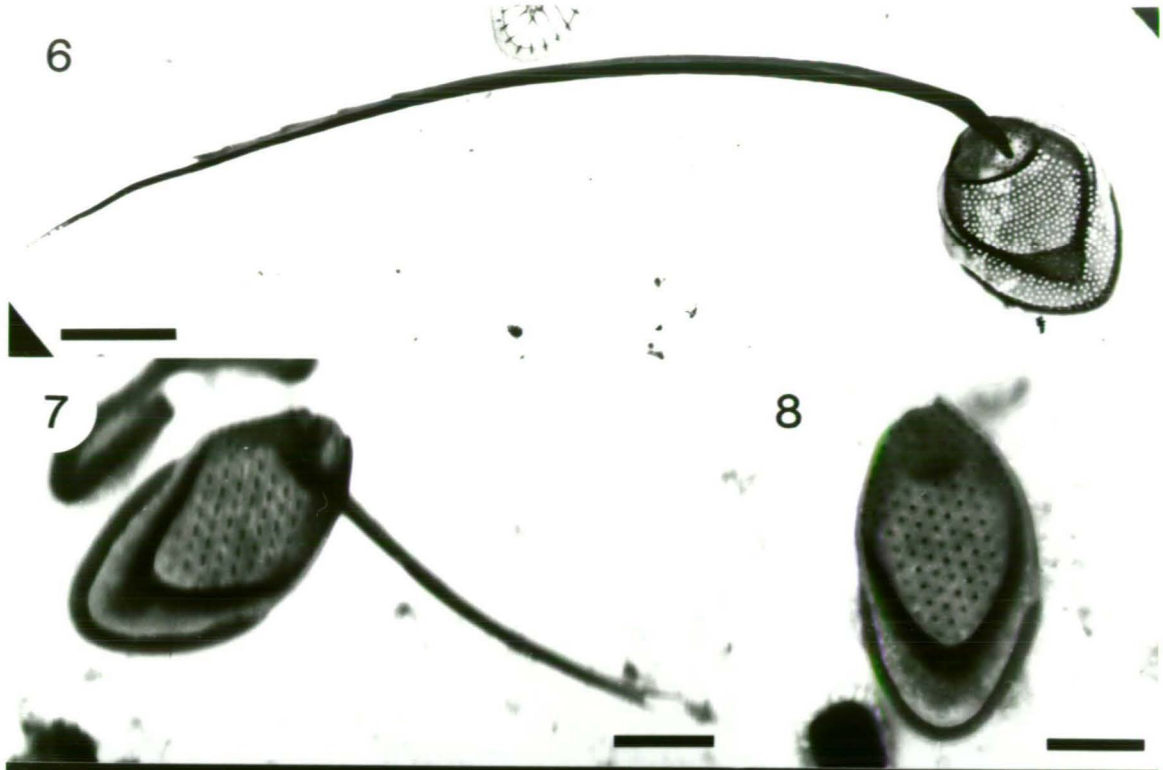
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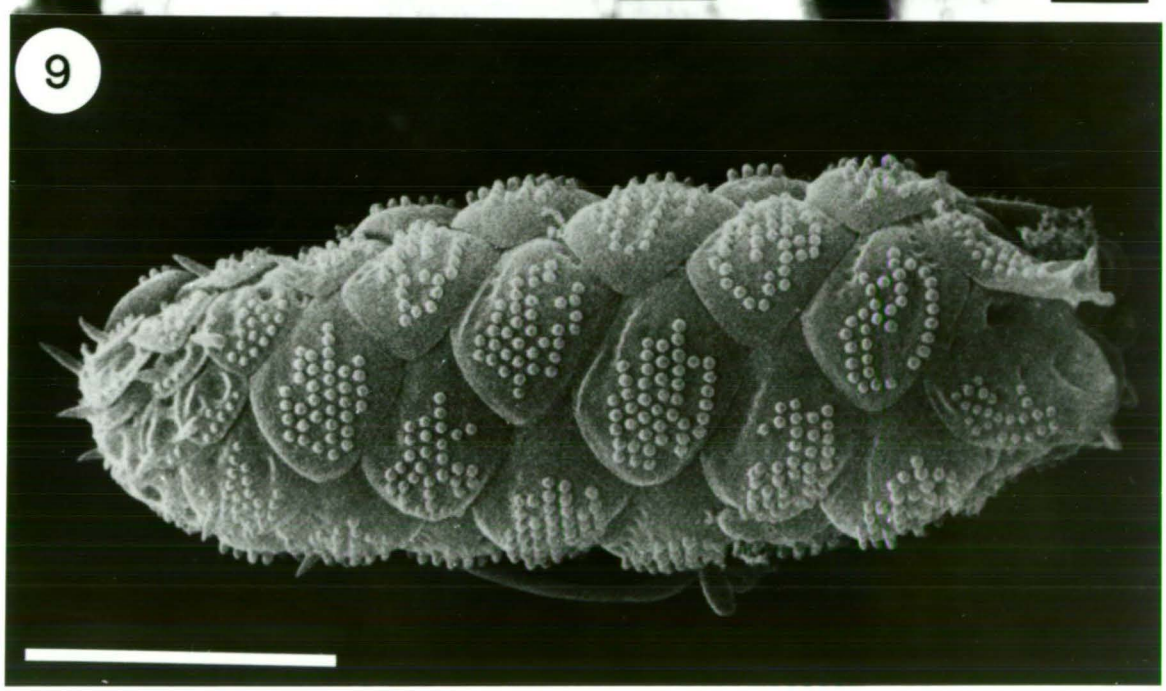
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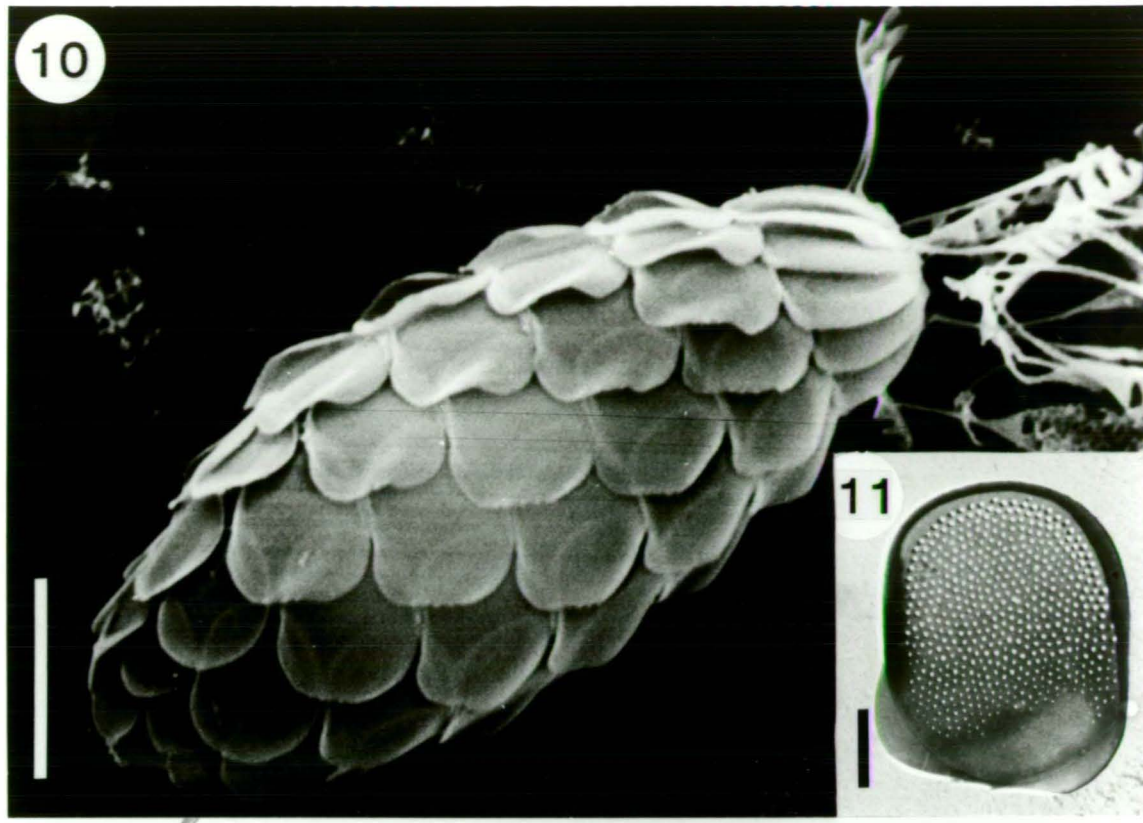
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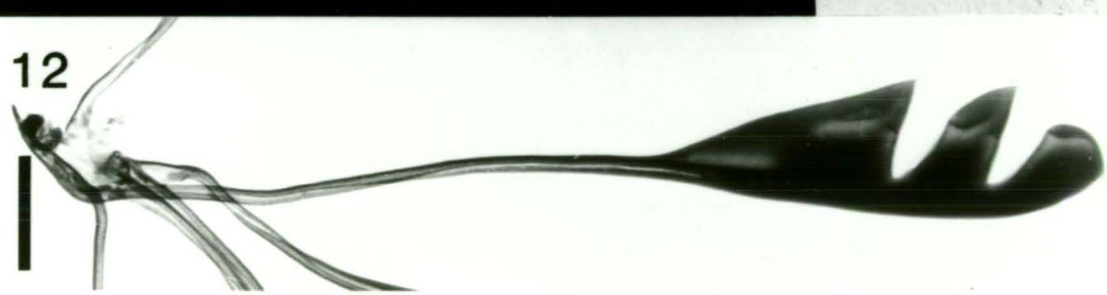
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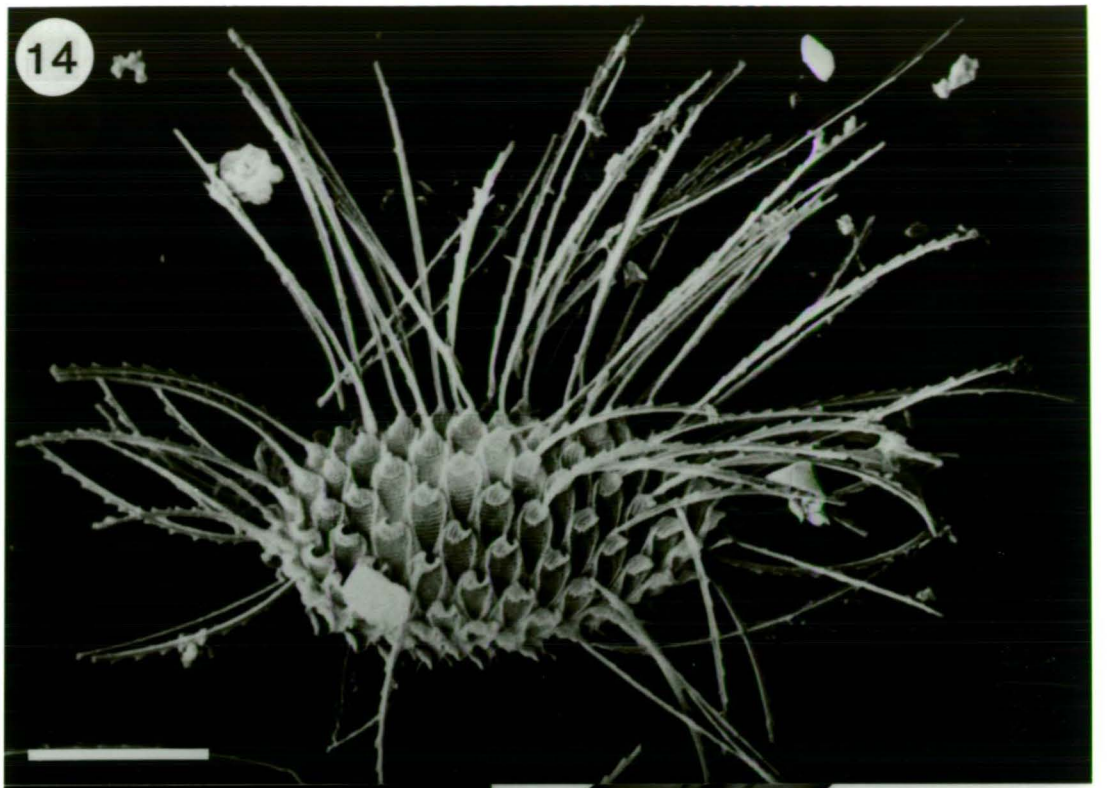
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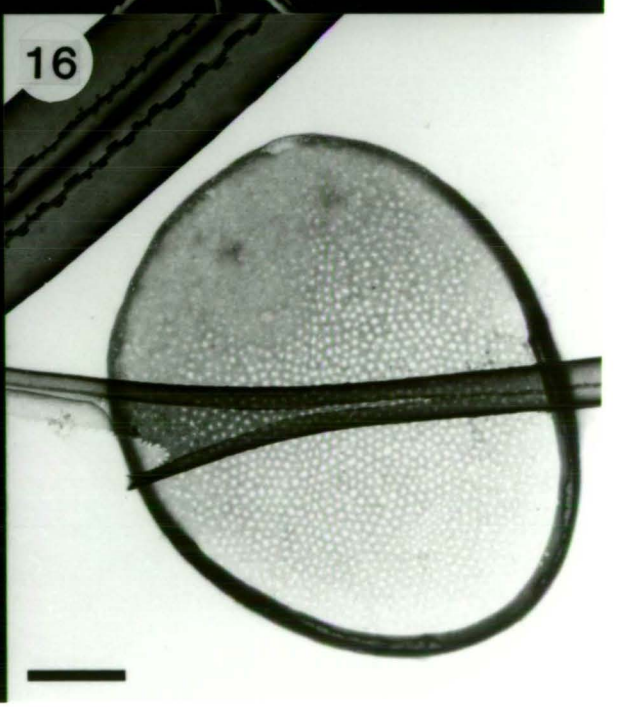
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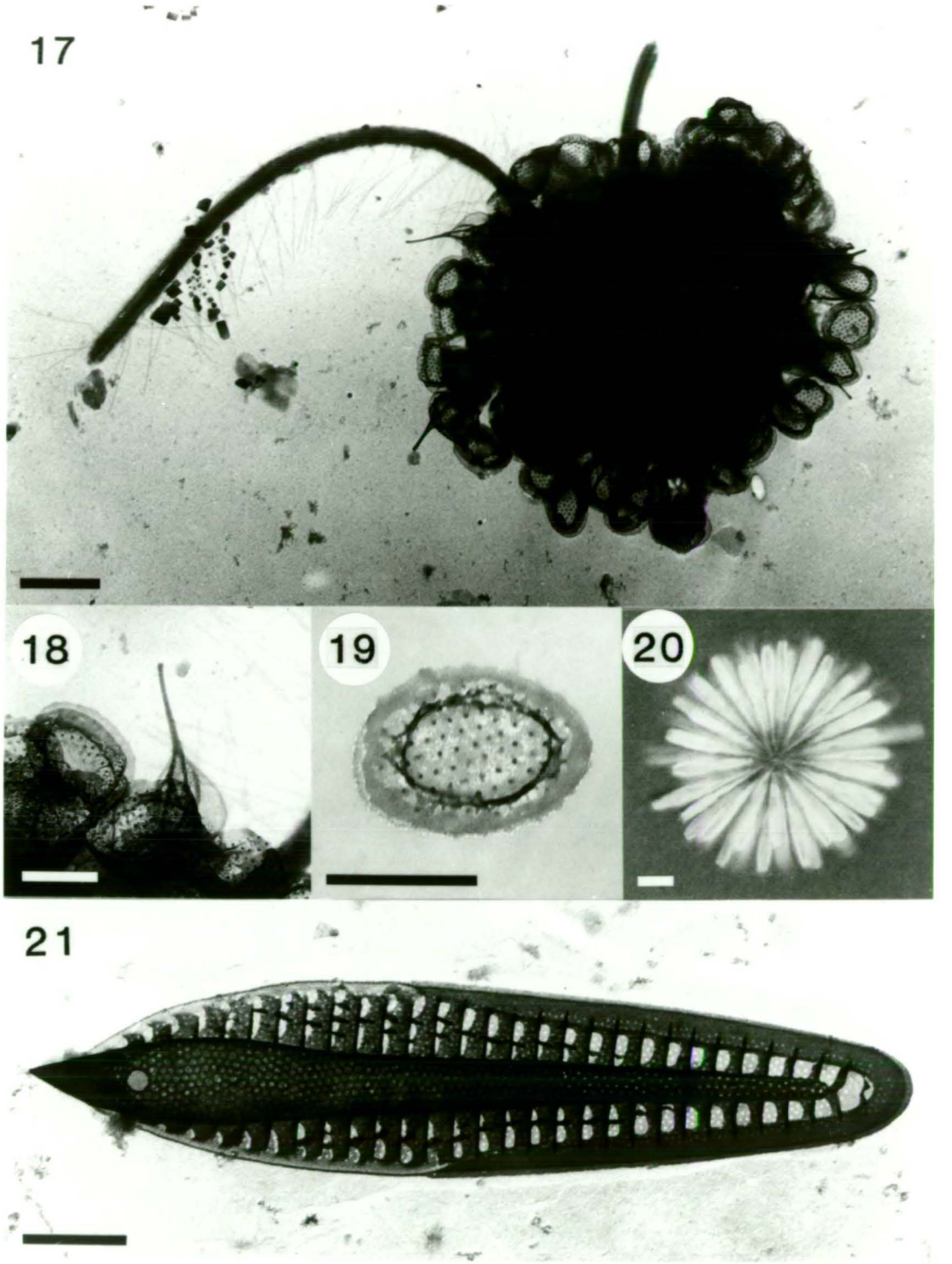
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Appendix X

CROOME, R.L. and TYLER, P.A. (1984e). A re-examination of Playfair's *Synura australiensis* and *Synura granulosa*. NORDIC J. BOTANY (Submitted).

A re-examination of Playfair's *Synura australiensis* and *Synura granulosa*

by

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Running title: *Synura australiensis* and *S. granulosa*

Abstract

Synura australiensis Playfair 1915 is described from light and electron microscopy. It has distinctive cell morphology but its scales are almost identical with those of S. petersenii Korsh. 1929. The relationships between S. australiensis, S. petersenii and S. granulosa Playfair 1915 are discussed. We conclude that S. petersenii is probably synonymous with Playfair's S. granulosa.

Introduction

Playfair (1915) described two new species of Synura from near Lismore, New South Wales, Australia. One of them, S. australiensis, he described as a "very beautiful and distinct species", depicting very elongate cells with truncate apices. The other, S. granulosa, he described as being the most common Synura of Australia. During an Australia-wide survey of the Mallomonadaceae we have found the distinctive S. australiensis to have a wide but localized distribution, but have found the most common Australian taxon to be S. petersenii.

Materials and Methods

Samples were collected with a 25 μm pore plankton net and fixed with formalin. Specimens for scanning electron microscopy (SEM) were placed on polylysine-coated glass coverslips (Marchant & Thomas, 1983), dehydrated in an acetone series, critical point dried using CO_2 as the intermediary fluid, sputter-coated with gold, and viewed using a Philips 505 SEM. Material for transmission electron microscopy (TEM) was put onto Formvar-coated copper grids, shadowed with platinum/palladium (2 parts to 1) and viewed using an Hitachi H-300 TEM.

Observations

We observed entire colonies of S. australiensis only from Kulukuluku Billabong, Northern Territory. Their diameter is approx. 130 μm. By light microscopy, the elongate cells have the truncate ends and smooth outline (Fig. 3) described by Playfair. Individual cells (Fig. 1), measuring 34-70 x 6.0-7.5 μm, were found in the northern tropics (Kulukuluku Billabong), south-eastern Australia (Lake Hume, N.S.W.-Victoria border) and the cool temperate Tasmania (Lake Leake),

but not in numerous other samples from throughout Australia. The scales (Figs. 2,4,5), 7.3-8.2 x 1.0-1.8 μm, are elongate, those from the stipe of the cell being narrower (Fig. 5). Apart from the more elongate shape, both types of scale match almost exactly the detailed description of S. petersenii given by Petersen & Hansen (1956).

S. petersenii occurs commonly throughout Australia. In South Australia, in particular, it causes taste and odour problems in urban water supplies, necessitating costly dosing of reservoirs with algicides.

Discussion

Our observations of the cells of S. australiensis by light microscopy agree perfectly with those of Playfair. By electron microscopy the body scales are identical, except for their more elongate shape, with those described in great detail for S. petersenii by Petersen & Hansen (1956). In the lakes in which we have found S. australiensis we have also found S. petersenii, but there has been no inter-gradation between the two in either cell shape or the length of the scales; S. australiensis is as distinct as it is distinctive.

Playfair also described S. granulosa from his Lismore samples, noting that it was "the common Synura of this country." From electron microscopy we know S. petersenii to be the most common Australian taxon, and by light microscopy our observations of S. petersenii are identical with those by Playfair of S. granulosa. We have examined Playfair's original collections by light microscopy, and found distorted cells of both S. australiensis and S. granulosa, but have been unable to locate scales for electron microscopy. In the absence of electron micrographs of Playfair's type material, and in view of the present problems in the classification of this group (Kristiansen, 1979), we are loth to take the obvious nomenclatural step of recognizing the precedence of

S. granulosa over S. petersenii until a thoroughgoing review of the genus is undertaken. For the same reasons we do not designate S. australiensis as a variety of S. petersenii at this time.

Acknowledgements

We thank the Australian Research Grants Scheme and the University of Tasmania for research grants, Dr. D.P. Thomas and Mr. A. Eastgate for assistance during electron microscopy, Dr. Hau U Ling for providing the light micrograph of S. australiensis (Fig. 3), and the National Herbarium of New South Wales for making available the type material of Playfair.

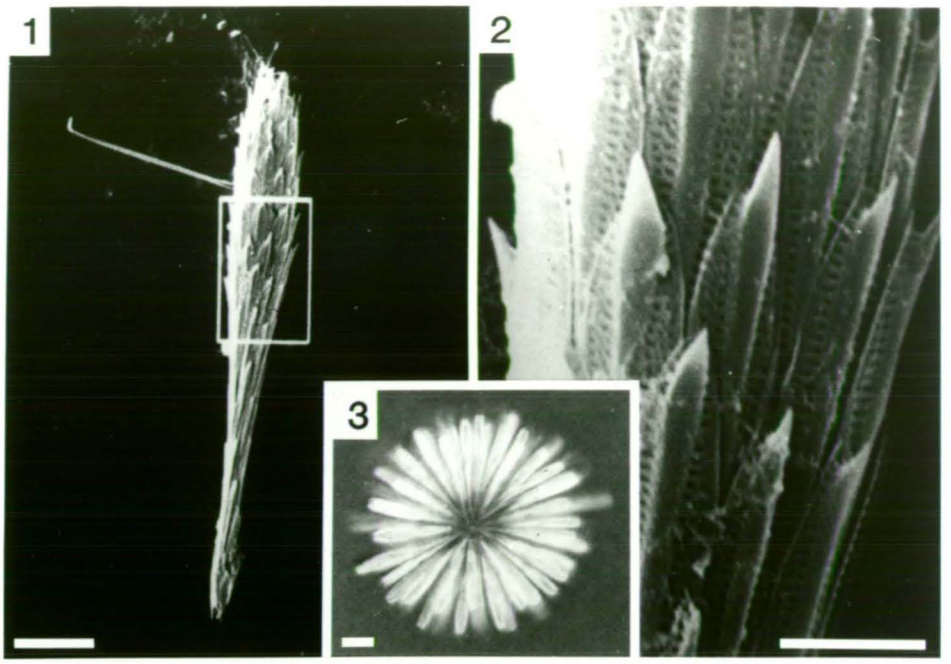
References

KRISTIANSEN, J., 1979. Problems in classification and identification of Synuraceae (Chrysophyceae).

MARCHANT, H.J. & THOMAS, D.P., 1983. Polylysine as an adhesive for the attachment of nanoplankton to substrates for electron microscopy. J. Micros., 131: 127-129.

PETERSEN, J.B. & HANSEN, J.B., 1956. On the scales of some Synura species. Biol. Medd. Dan. Vid. Selsk., 23: 1-27.

PLAYFAIR, G.I., 1915. Freshwater algae of the Lismore District: with an appendix on the Algal Fungi and Rhizomycetes. Proc. Linn. Soc. NSW., 40: 310-362.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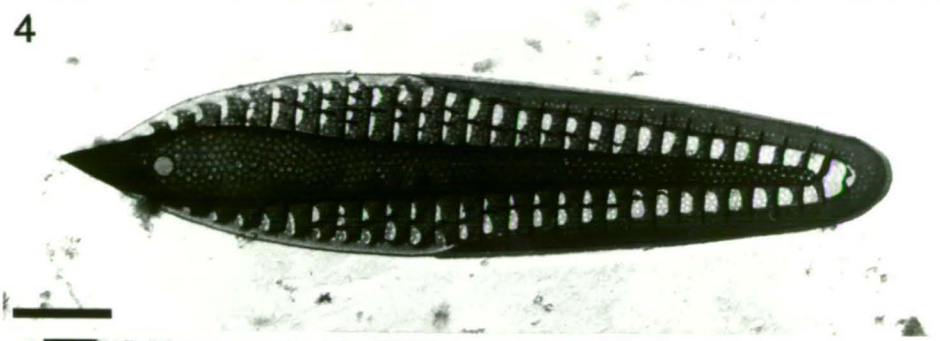
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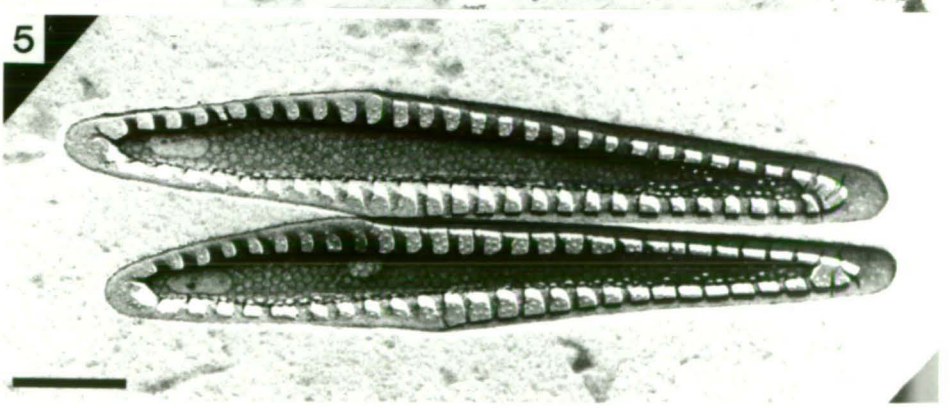
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